A STUDY OF SERUM LEVEL OF ISCHEMIA MODIFIED ALBUMIN IN ACUTE CORONARY SYNDROME

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Remark of the Guide:

The work done by **DR.R.FREETHI** on **"STUDY OF SERUM LEVELS OF ISCHEMIA MODIFIED ALBUMIN IN ACUTE CORONARY SYNDROME"** is under my supervision and I assure that this candidate will abide by the rules of the Ethical Committee.

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CERTIFICATE

This is to certify that dissertation titled "**A STUDY OF SERUM LEVEL OF ISCHEMIA MODIFIED ALBUMIN IN ACUTE CORONARY SYNDROME"** is a bonafide work done by **Dr.R.FREETHI** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2012 to 2015.

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DECLARATION

I, **Dr.R.FREETHI** hereby solemnly declare that the dissertation title "**A STUDY OF SERUM LEVELS OF ISCHEMIA MODIFIED ALBUMIN IN ACUTE CORONARY SYNDROME"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the Supervision and Guidance of my Professor and Head of the Department **Dr.N.Sasivathanam, M.D(Bio).,DGO,**, This dissertation is submitted to Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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CONTENTS

ABBREVIATIONS

WHO – World Health Organisation.

IHD – Ischemic Heart disease.

CHD – Coronary Heart disease

CVD – Cardio Vascular disease

IMA – Ischemia Modified Albumin

CK-MB – Creatine Kinase – MB isoform

LDH – Lactate dehydrogenase

AST – Aspartate transaminase

ECG – Electro Cardiogram

WHO – World Health Organization

MI – Myocardial infarction

TC – Total cholesterol

TAG – Tri acyl glycerol

HDL – High density lipoprotein

VLDL – Very low density lipoprotein

LDL – Low density lipoprotein.

STUDY OF SERUM LEVEL OF ISCHEMIA MODIFIED ALBUMIN IN ACUTE CORONARY SYNDROME:

ABSTRACT:

BACKGROUND:

Ischemic heart disease is the greatest single cause of mortality and loss of disability-adjusted life years worldwide. Early identification and management of patients with acute myocardial infarction reduces the mortality phenomenally. Along with ECG and other clinical parameters, cardiac biomarkers do have an important role in the early diagnosis and management of patient with ACS. This study purposes to study the role of Ischemia modified albumin as a early marker of myocardial ischemia.

AIM AND OBJECTIVE:

To measure Serum Ischemia Modified Albumin within 6 hours of onset of chest pain and to correlate the IMA values with CK-MB, LDH, TGL, T-CHOL, HDL, LDL & VLDL.

MATERIALS AND METHODS:

The study was conducted in Thanjavur Medical College Hospital. 50 patients with symptoms of acute coronary syndrome presented within 6 hours of onset of pain in the causality with ECG findings correlated and were taken as subjects . 50 age and sex matched controls were taken as control group. Blood

samples were subjected to estimation of IMA (ACB test) , CKMB, LDH, SGOT and lipid profile.

RESULTS:

The mean value of IMA for control and study group were 37.67±14.74 and 115.07±17.55 respectively . The Mann Whitney U score was .000 and p value .000 (<0.01) . Spearman correlation showed significant correlation of Ischemia modified Albumin with CK-MB, lipid profile and LDH.

CONCLUSION:

The results of the present study confirm the findings of previous studies, that reported that the Albumin Cobalt colorimetric assay distinguishes myocardial ischemic patients from non ischemic patients ($p<0.001$). IMA assay presents a quantitative accurate laboratory determination of the occurrence of an Ischemic myocardial event including angina of various types. Measurement of Ischemia Modified Albumin levels diagnose Acute Coronary Syndrome in patients with ongoing myocardial ischemia in Emergency Department. Measuring IMA along with ECG and other markers improves the diagnostic sensitivity of the method.

INTRODUCTION:

Acute coronary syndrome (ACS) is an umbrella term for a wide spectrum of clinical signs and symptoms suggestive of myocardial ischemia.[1] Myocardial ischemia denotes decrease in oxygen and nutrient supply to the cardiac myocytes due to inadequate blood perfusion. Myocardial infarction refers to cell death due to prolonged ischemia.[2]

Ischemic heart disease is the greatest single cause of mortality and loss of disability-adjusted life years worldwide.[3] Further a substantial portion of this burden falls on low- and middle-income countries. Following are the factors which underline the importance of developing newer diagnostic and therapeutic strategies for this disease:

1. Epidemiology of the disease
2. Economical , social and legal liabilities for clinicians
3. Lethal consequences of the disease.[4]

Notwithstanding this, early identification and management of patients with acute myocardial infarction reduces mortality phenomenally[5]. The traditional clinical approaches such as detailed history, careful physical examination and ECG findings are helpful in stratifying the patients but it seems to be inadequate in early definitive diagnosis in many of the cases.[6 ,7] Along with ECG and other

clinical parameters, cardiac biomarkers do have an important role in the early diagnosis and management of patient with ACS.

The term 'biomarker', an abbreviation for "biological marker" is defined as a characteristic substance in serum or any body fluid which can be objectively measured and recognised as an indicator of normal biological processes, an important component or event of the pathogenic processes or pharmacological responses to therapeutic interventions.[8] While a single biomarker sometimes covers this role in certain diseases, in multifactorial diseases like coronary artery disease (CAD), the task of identifying the biomarkers is an ongoing task.[9]

ACS starts when there is disruption of atherosclerotic plaque in the coronary artery, causing occlusion of the vessel preventing myocardial perfusion and stimulating aggregation of platelets and formation of thrombus. Data from recent studies emphasises that the rupture of a vulnerable plaque which is unstable and its associated inflammatory changes as the reason for impaired perfusion rather than impaired blood flow in response to narrowing of the arteries due to thickening of the preformed plaque.[10,11,12]

The contractility and the electrical stability of the myocardial cells need oxygen and adenosine 5β-triphophate (ATP). As the myocardial perfusion decreases gradually the following changes or phases takes place.

1. **ISCHEMIC PHASE**: In this phase, both aerobic and anerobic metabolism take place in the cells.
2. **INJURY PHASE**: When there is continuous decrease in perfusion, aerobic metabolism fails and subsequently anaerobic metabolism also becomes significantly reduced.
3. **NECROSIS OF THE MYOCARDIAL CELLS**: Myocardial necrosis with irreversible damage proceeds when perfusion is not restored in about 20 minutes.[13]

The cardiac biomarkers currently in wide use in clinical practice like creatine kinase (CK) and its fraction CK-MB, troponins, myoglobin and natriuretic peptides are markers of myocardial necrosis which happens at the downstream of the pathophysiology of ACS.

Recent investigations are directed towards analysing components involved in processes upstream from necrosis, such as components released during ischemia, components of plaque destabilisation and rupture, factors of thrombosis, molecules of inflammation and acute phase reactants for earlier assessment and risk stratification and indexing them under “biomarkers”.

Apart from being sensitive, an ideal biomarker of myocardial injury should be cardiac specific, easily measurable and accurate, thereby influencing therapy and improving patient outcome.

Ischemia modified albumin(IMA) is a U.S. Food and Drug Administraion approved biomarker for acute ischemia. The N terminus of albumin is damaged when exposed to ischemic conditions, making it unable to bind metals thus enabling it to be measured using albumin cobalt binding test.[14,15,16] The levels of IMA increase within minutes of onset of ischemia- implicating it in the early detection of acute ischemia before the onset of necrosis.

This study purposes to study the serum levels of Ischemia modified albumin as an early marker in myocardial ischemia.

AIM:

To measure the serum levels of Ischemia Modified Albumin in paitents with ACS presenting within 6 hours of onset of chest pain.

OBJECTIVES :-

(1) To correlate the IMA values with CK-MB

(2) To correlate the IMA values with LDH

(3) To correlate the IMA values with other markers of atherosclerosis like

- Total cholesterol
- Triacylglycerol
- High density lipoprotein
- Low density lipoprotein
- Very low density lipoprotein

(4) To prove the use of IMA as an early marker of myocardial ischemia.

REVIEW OF LITERATURE:

Ischaemic heart disease (IHD) is a clinical syndrome which occurs due to underperfusion caused by reduced blood supply to the cardiac musculature.[17] Impairment or disturbances in the coronary blood flow due to atherosclerotic narrowing is the commonest cause but can also occurs due to arterial spasm. (Warrell et al, 2004).

The term "acute coronary syndrome" (ACS) includes a range of thrombotic coronary artery diseases, including unstable angina (UA) and both ST-segment elevation (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI).[18]

EPIDEMIOLOGY:

Over the last decade, cardiovascular disease (CVD) stands for the single largest cause of death worldwide. According to World Health Organisation (WHO) global report, in 2004, 17 million deaths and 151 million disability – adjusted life years (DALY) were caused by CVD. The prevalence of risk factors for IHD has increased in the developing world as a result of urbanisation and it has been predicted that the prevalence of IHD is rising and IHD is most likely to become the most common cause of death worldwide by 2020. [20,21]

APPLIED ANATOMY

Heart is a hollow muscular organ located in the mediastenum resting on the diaphragm. It is enclosed by a covering called pericardium. The cardiac walls comprises of three layers - Peicardium, Mesocardium and Endocardium, from external to internal. It consists of four chambers- two atrias and two ventricles. Of these ventricles especially left ventricle are thicker than the atrias.

BLOOD SUPPLY:

The right and left coronary arteries originating from the aortic sinuses provides blood flow and nutrient perfusion to the heart. These arteries gives off subsequent larger to smaller branches from epicardium to endocardium of which endocardium is the layer most susceptible to ischemia because its perfusion relies on the smallest branches.

The myocardium is made of bundles of striated muscle fibers the alternate contraction and relaxation of which enables the functioning of the heart. These fibers contains proteins like cardiac specific contractile proteins, regulatory proteins, enzymes and proteins for energy use. They are called actin and myosin troponins, and myoglobin, creatine kinase, lactate dehydrogenases respectively and these proteins are used as cardiac markers.

CARDIAC METABOLISM

The mechanical pumping and the regulation of intracellular and trans-sarcolemmal ionic movements and concentration gradients require continuous supply of oxygen approximately 15% of that of the organism and energy in the form of ATP.

The energy need of the heart depends on the following:

- Developing tension,
- The rate of contractions and
- The level of contractility . [21]

ATP production in the cardiac muscle takes it source mainly from glucose and free fatty acids which are supplied from plasma, cell store (glycogen) and from lipolysis of adipose tissue respectively. There is a reciprocal relation between the utilization of these two principal sources of acetyl CoA in cardiac muscle. Both glucose and free fatty acids are broken down into acetyl coA inside the cytoplasm and mitochondria respectively which eventually enters the TCA cycle inside the mitochondria to yield the energy source ATP . these ATP enters into the cytoplasm and use for various physiological purposes.

In the fasted, resting state, the main source of acetyl COA (70%) will be the circulating FFA and their uptake are high. In the fed state, blood glucose and

insulin, glucose oxidation increases and FFA oxidation decreases subsequently. Factors which increases the myocardial glucose uptake from glycogenolysis and glycolysis are increased cardiac work, the administration of inotropic agents, hypoxia, and mild ischemia.

During stress the adrenergic stimulation cause increased circulating levels of FFAs and its metabolism in favour of glucose. But in despite the breakdown of more glycogen and glucose breakdown , only lactic acid is produce from glucose (anerobic glycolysis) which donot enter TCA cycle. Moreover , ischemia inhibits the cytoplasmic enzyme pyruvate dehydrogenase. As a result less ATPs are produced by these anaerobic glycolysis than aerobic glycolysis which leads to production of pyruvate and subsequently oxidized to CO_2.[22]

The sequence of events when adrenergic stimulation superimposed on myocardial ischemia are.

- Adrenergic stimulation and severe ischemia
- Reduce oxidative phosphorylation
- High concentrations of circulating FFAs
- Wastage of ATP
- Decreased ATP content
- Dysfunctional myocardial contraction.

Creatinine phosphate is the main source of energy which will be in equilibrium with ATP. In reduced energy state , creatine phosphate stores declines first.[23]

Conditions requiring increased myocardial energy.

1. Hypertrophy of cardiac muscle,
2. Fibrosis,
3. Increased heart rate,
4. Increase in wall tension due to ventricular dilatation,
5. Intracytoplasmic calcium [Ca^{2+}] increase

Creatine phosphate is the source of energy stored in the myocardium. This is in balance with ATP which is the immediate source of energy. CP stores are the first to decline when there is reduced availability of energy. There is an elevated need for myocardial energy when there is hypertrophy of the cardiac musculature, fibrosis, increased heart rate, dilation of the ventricles due to increase in the tension of the cardiac walls and increased Ca^{2+} in the intracytoplasmic compartment. Ischemia can be worsened or cardiac failure can be precipitated when there is mismatch between the demand and production of myocardial ATP which can occur in the event of diminution of coronary reserve due to impeded coronary flow culminating from as a result of abnormal microcirculation and/or obstruction.

FACTORS INFLUENCING MYOCARDIAL O2 SUPPLY AND DEMAND[24]

I.MYOCARDIAL OXYGEN SUPPLY:

Coronary blood flow:

1. Coronary perfusion pressure
2. Coronary vascular resistance
3. External compression
4. Intrinsic regulation

Local metabolites

1. Endothelial factors
2. Neural innervations

II. MYOCARDIAL OXYGEN DEMAND

1. Wall stress
2. Heart rate
3. Contractility

III. SECONDARY PRECIPITANTS OF MYOCARDIAL ISCHEMIA:

1. INCREASED MYOCARDIAL OXYGEN DEMAND.

Fever

Thyrotoxicosis

Tachycardia

Malignant hypertension

Pheochromocytoma

Aortic stenosis

High output status

Pregnancy, drugs: cocaine, amphetamine.

2. DECREASED OXYGEN SUPPLY

Anemia

Hypoxemia

Carbon monoxide poisioning

Poycythemia

Hyperviscosity syndromes

CLINICAL PRESENTATIONS OF IHD

The following clinical presentations can be encountered due to reduction in the blood flow to the cardiac musculature. [25]

- Angina pectoris also known as Chronic stable angina
- Myocardial infarction and unstable angina (Acute coronary syndromes).
- Silent myocardial ischaemia (Chronic ischaemic heart disease)
- Sudden cardiac death

ANGINA PECTORIS:

This is a clinical presentation of Ischemic Heart Disease consisting of symptoms of discomfort or pressure in the precordial area which occurs as a result of temporary disruption of blood flow to the cardiac musculature not progressing to overt infarction. This is characteristically exacerbated by physical exercise and mental stress while rest and nitroglycerin used sublingually relieves it.[26]

Angina pectoris results when there is a mismatch between the demand for O_2 which increases due to increased workload of the heart and the ability of the coronary arteries to supply the same as a result of narrowing. Atherosclerosis, coronary artery spasm or in rare instances, embolism of the coronary artery can cause such narrowing of the coronary artery. While angina can be precipitated by acute coronary thrombosis which usually causes myocardial infarction.

Myocardial O_2 demand usually rises with increase in mainly heart rate, contractility and tension of the cardiac wall during cardiac systole. A narrowing of the coronary vessels in this scenario results in the exaggerated mismatch between supply and demand and results in angina. The angina is relieved with rest since the demand no more exists.

Conditions like systemic hypertension, stenosis of the aortic valve, aortic valvular insufficiency and hypertrophy of the cardiac muscle increase the work load of the heart. Irrespective of the presence or absence of atherosclerosis, these conditions can precipitate angina. These disorders also increase the myocardial mass thereby decrease the diastolic flow and effectively cause reduction in the relative myocardial perfusion.

PRINZMETAL ANGINA:

Prinzmetal's or Prinzmetal angina which is also known as variant angina, angina inversa, or coronary vessel spasm) is another type of presentation of myocardial ischemia typically characterised by chest pain at rest (angina) usually occurs in cycles. In contrast to angina induced by narrowing of blood vessels due to atherosclerosis this type of pain is due to vasospasm, a narrowing of the coronary arteries which is caused by contraction of the smooth muscle tissue in the vessel walls. [27]Abnormal constriction of the conductance vessels can cause severe ischemia in Prinzmetal’s angina epicardial coronary arteries are capable of constriction

and relaxation, in healthy persons they serve as conduits and are referred to as conductance vessels, while the intramyocardial arterioles normally exhibit changes in tone and are therefore referred to as resistance vessels . Abnormal constriction of the conductance vessels can cause severe ischemia in Prinzmetal's angina

ISCHEMIC CARDIOMYOPATHY

Patients with IHD can also present with cardiomegaly and heart failure secondary to ischemic damage of the left ventricular myocardium that may have caused no symptoms prior to the development of heart failure; this condition is referred to as ischemic cardiomyopathy.

ACUTE CORONARY SYNDROME:

The term ACS has been introduced as a new clinical phenotype. It adequately describe the full range of clinical manifestations resulting from atherosclerotic coronary plaque, fissuring or rupture leading to myocardial ischemia after incomplete or complete coronary artery thrombotic occlusion.

SUSPECTED ACS:

When signs and symptoms on admission suggest underlying myocardial ischemia but definitive objectives still lacking. It is the combined evidence of ECG, and cardiac bio markers that refines the diagnosis.

Whenever the perfusion in the coronary arteries decreases due to atherosclerosis, there is a fall in the O_2 tension in the myocardium. This causes temporary disturbances in the biochemical, electrical and mechanical milieu of the heart musculature. Therefore normal muscle contraction and relaxation is instantly disrupted due to sudden development of intense ischemia which may occur in total or subtotal coronary blockage. The subendocardium of the heart has poor vascular perfusion and the ischemia is more severe in this portion of the wall of the heart.

The disruption of coronary vessel atherosclerotic plaque results in aggregation of platelets and formation of thrombus inciting the chain of events culminating in ACS. The thrombus occluding the vessel with subsequent reduced myocardial perfusion and not the coronary artery narrowing which is in response to thickening plaque, as believed in the past, is the primary reason for impending blood flow that leads to ischemia. Further, an unstable, vulnerable plaque rupture with added inflammation, has also been stipulated as cause of decreased myocardial perfusion.[28]

CLASSIFICATION

ACS can be classified on the basis of changes in ECG and the increase or decrease of cardiac biomarkers. Further, classifying ACS on the basis of ST segment elevation as NSTEMI and STEMI is beneficial since they differ in the treatment required and prognosis .

1. STEMI - **ST-segment elevation MI** or transmural MI
2. NSTEMI - **Non–ST-segment elevation MI** or subendocardial MI
3. Intermediate syndrome **Unstable angina** or acute coronary insufficiency or preinfarction angina

UNSTABLE ANGINA (also called as preinfarction or acute coronary insufficiency or intermediate syndrome) characterised by:

- Prolonged angina at rest (existing more than 20 minutes)
- New onset of angina which has occurred newly with the severity of class 3 in the Canadian Cardiovascular Society (CCS)
- Recurring and increasing angina which was already diagnosed recurring with increasing frequency, severity and duration or lower threshold (eg, increased to at least CCS class 3 or by $\geq$ 1 CCS class or 2).

Transient ECG changes like depression or elevation of ST segment, or depression of T wave can occur in unstable angina. But it is only transient. If at all cardiac markers are elevated it is only a slight increase in the high sensitivity

Troponin tests (hs-cTn), whereas markers like CK are not elevated. MI, arrhythmias or even sudden death can occur following unstable angina. [29,30]

NON–ST-SEGMENT ELEVATION MI (subendocardial MI or NSTEMI) This type of myocardial infarction will be characterised by increased levels of cardiac markers in serum like troponin I or troponin T and CK but without the appearance of Q waves or acute ST-segment elevation. And also ECG changes like depression of ST-segment, inversion of T-wave , or both may be present.

ST-SEGMENT ELEVATION MI (STEMI or transmural MI) This type of myocardial necrosis are characterised by the changes in ECG which shows ST-segment elevation usually not rapidly reversed by nitroglycerin or evidenced by the presence of new left bundle branch block and Q waves may possibly present. Further elevation of specific Cardiac markers like troponin I or troponin T and CK are found to be elevated.

MI also can be classified into 5 types based on etiology and circumstances:

- Type 1: Spontaneous MI as a result of ischemia caused by prime coronary event presenting after rupture of an atherosclerotic plaque or erosion or fissuring or coronary dissection)
- Type 2: Ischemia due to increased O_2 demand (eg, hypertension), or decrease in oxygen supply caused by spasm of coronary artery or embolism or hypotension or arrhythmia

- Type 3: Sudden unexpected cardiac death
- Type 4a: Myocardial infarction eventually with Percutaneous Coronary Intervention (PCI)
- Type 4b: Thrombosis of documented stent presenting with myocardial infarction
- Type 5: Myocardial infarcation after Coronary Artery Bypass Grafting (CABG)[31,32]

RISK FACTORS FOR ACS: [33]

UNMODIFIABLE RISK FACTORS ARE:

- Race
- Age
- Male gender
- Genetic factors
- Low birth weight.
- Family history.
- Previous history of CVD

PARTIALLY MODIFIABLE RISK FACTORS ARE:

- Post menopausal oestrogen deficiency.
- Diabetes

- Chronic renal diseases.
- Infection.
- Stress.
- Personality
- Socioeconomic status
- Microalbuminuria
- Chronic inflammatory.
- Lipoprotein (a).

MODIFIABLE RISK FACTORS ARE:

- Metabolic
    - Hypercholesterolemia
    - Inflammation
    - High plasma fibrinogen
    - Low HDL
    - Hyperinsulinemia and insulin resistance.
    - Hyper triglyceridemia
    - Small dense LDL
    - Visceral obesity
    - Hypertension.
    - Hyperhomocysteinaemia

- Behavioural
    - Smoking
    - Low physical activity.
    - Alcohol intake > 89g/day

AGE Vs ACS:

Post mortem evidences have demonstrated fatty streaks and also advanced lesions in the second decade of life which usually do not become clinically evident until the fifth or sixth decades. Experimental models of atherosclerosis showed that some of the lesions are reversible to some extent. Increasing prevalence of atherosclerosis among young people may be partly due to increase in cholesterol concentration and other coronary risk factors as the age advances. The relative risk with increasing cholesterol is steeper in young. The prevalence of coronary and aortic atherosclerosis increases with age.[34]

GENDER:

Generally many studies have shown that women presenting with acute ischemic syndromes are most likely to be older than men and also more likely to have a history of any medical problem like hypertension or diabetes or angina or congestive heart failure. The reason is that they are less likely to be smokers or to have had a history of prior infarction. Women have more percentage of HDL cholesterol than men which is due to the action of oestrogens and it seems to be

vascular protective also. Post menopausal periods are associated with a rise in plasma LDL cholesterol ,fall in HDL cholesterol and increasing visceral adiposity increases the risk for CHD . And also ST elevations are significantly lower in females than in males, but unstable angina was significantly higher than that of men. Women were shown to be less likely associated with occlusive thrombus even after adjusting for baseline differences that is ST elevation with infarction. But the relative protection in female gender becomes lower if they have diabetes.[35,36,37,38]

FAMILY HISTORY:

A family history of premature CVD in an important risk factor. Familial tendency to atherosclerosis and ACS are almost certainly polygenic. Family history is particularly important contributor to risk in men in the lowest quintiles for calculated CHD risk . In women, age-adjusted risk is increased almost three fold if either parent has CHD before 60 years of age. Moreover risk factors like hypertension and diabetes mellitus are more inclined to genetic susceptibility. [38]

DIABETES MELLITUS:

Many patients admitted in hospital with with acute coronary syndromes (ACS) were associated with hyperglycaemia. Hyperglycemia has been shown to predict the survival and the risk of complications in both diabetic and non-diabetics. When compared to patients admitted with normal glucose

concentration there is 70% increase in the relative risk in patients admitted with plasma glucose more than 180mg/dl. Both diabetes and impaired glucose tolerance are important risk factors for CVD and epidemiological data have led to the notion that diabetes confers a similar risk of a cardiovascular event to a prior myocardial infarction. Diabetic patients with high haemoglobin A1c is possibly related to an increased CVD risk.[40]

In a study done by Foo et al , a near-linear relationship was demonstrated between higher levels glucose levels on admission with the rate of left ventricular failure and cardiac death due to ACS among 2127 patients.

Meier et al also have demonstrated the occurrence of higher long-term mortality rates with larger infarct size among hyperglycaemic AMI patients with or without diabetes. [42]

Stranders and Wahab et al have also shown the associated risk of admission hyperglycaemia with AMI patients devoid of previously known diabetes.

METABOLIC SYNDROME:

The clustering of several coronary risk factors (high triglycerides, low HDL. Obesity, hyperuricemia, hyperinsulinemia and hypertension) has been known for several decades . Reaven proposed the existence of a syndrome with a common underlying metabolic defect. At any particular level of coronary risk based on the Framingham and PROCAM algorithms, patients with metabolic syndrome appear to have higher event rates than predicted.[43]

CHRONIC KIDNEY DISEASE(CKD):

CKD patients are prone to have high prevalence of arteriosclerosis and large arteries remodeling due to vascular calcification. Reduced Glomerular Filtration Rate (GFR) have linear relationship with a high prevalence of CVD risk factors and a higher prevalence of CVD surrogates and clinical CVD. More recently, the extent of demonstrable angiographic coronary disease reflects the level of kidney function in patients with CKD. For example, women with chest pain undergoing angiography, an increased creatinine of 1.2 to 1.9 mg/dL is an self-determining predictor of significant angiographically proved coronary disease, with luminal narrowing of 50%.[44]

STRESS:

Mental stress is one of the principle precipitating factor for angina and myocardial ischemia in patients with underlying coronary artery disease. Myocardial oxygen demand is increased by sympathetic activation which is characterised by tachycardia ,high blood pressure and increase in myocardial contractility which further increases the myocardial oxygen demand. Although stress induces increased blood flow, s in the presence of atherosclerosis there will not be adequate increase in regional coronary blood flow inspite of increase in the blood pressure- heart rate product and plasma norepinephrine levels.[45]

PERSONALITY:

Type A behaviour increases the risk of coronary heart disease twice than in otherwise healthy men. In 1950s it was shown as an significant risk factor in coronary Disease by cardiologist Meyer Friedman and his coworkers. Type A personalities are charactised by set of features of being insecure about one's status impatient, hostile , time conscious, highly competitive, and aggressive and incapable of relaxation.[46]

LIPIDS AND LIPOPROTEINS:

The association between plasma lipids and coronary heart disease is positively correlated in many studies and was continuous , exponential and showed no threshold , even at low concentrations. Also the distributions of plasma total cholesterol concentrations among patients with and without CHD overlap to a considerable degree. Plasma apolipoprotein B (apo B) concentrations are more discriminating and a few clinical studies have used apo B concentrations as the basis for treatment.

An inverse relationship between plama HDL concentrations and CHD risk has also been demonstrated in various studies. Clinical experiments on protective effects of HDL have been supported by animal models, in which expression of human apo-AI has been shown to inhibit the development of atherosclerotic lesions. The beneficial effect of HDL may in part be due to its antioxidant properties associated with an intrinsic paraxonase I (PON I) activity.[47] Low PON I activity is an self-sufficient predictor of new coronary events and its

polymorphism is reported to be associated with increased lipid peroxide hydrolysis. Recent data do support a role for triglycerides as an independent risk factor. Concentrations of plasma triglycerides above approximately 1.7mmol/L are associated with the formation of the more atherogenic , small , dense LDL and also it has proatherogenic effects by promoting a procoagulant state, being associated with enhanced factor VII activity.[48]

LIPOPROTEIN (a):

Apolipoprotein(a) gene determines the plasma levels of Lp(a) and its variability genitically . Therefore apolipoprotein(a) gene appears to be ideal for use in Mendelian study of randomization for evaluating the relationship between permanent genetically elevated levels of plasma Lp(a) and CVD. (18) This helped to establish a direct relationship between atherosclerosis due to increased LDL cholesterol levels and CVD.

THOMBOGENESIS AND CLOTTING FACTORS:

Thrombin utilises fibrinogen as the substrate for the final step in cascade of coagulation. It is also necessary for platelet aggregation, modulation of endothelial function to promote smooth muscle proliferation. A recent systemic review has shown that plasma fibrinogen concentration is moderately strongly associated with CHD. The relationship between plasma fibrinogen and coronary risk may underlie the positive association between plasma viscosity and CHD.[49]

The tissue factor release is caused by the endothelial injury which in turn, activates the intrinsic clotting cascade. Platelet activation and aggregation are crucial processes in atherothrombogenesis, and platelet reactivity has been reported to be elevated in subjects with diabetes and unstable angina. There exists a balance between the formation of clot and its inhibition and dissolution by factors such as proteins C and S and plasmin. The effectiveness of the fibrinolytic system depends on the balance between tissue plasminogen activator (tPA) and inhibitors of plasminogen activator converts plasminogen to plasmin, which acts on fibrin causing clot dissolution. This process is inhibited by PA-I , high concentrations of which are associated with high risk for reinfarction. Evidence from Framingham study indicates that plasma PA –I concentrations rise with increasing systolic and diastolic pressure. Atherosclerotic leions from diabetic subjects have been shown to contain high concentrations of PA- I and plama PA- I is strongly associated with several CHD risk factors including body mass index (BMI), lipids and alcobol intake and these effects appear to be cumulative.

Apolipoprotein(a) is a glycoprotein that has structural homologies with plasminogen. It is attached to apo B by a disulphide bond and in some individuals, comprises the major cholesterol rich lipoprotein. The structural similarities between apo(a) and plasminogen have led to the proposition that Lp(a) inhibits plasmin activity , leading to a prothombotic state. Plasma concentration of Lp(a) are largely genetically determined, but can be modified

to a limited degree, by dietary fatty acids, oestrogen and lipid lowering agent like nicotinic acid.

HYPERINSULINEMIA AND INSULIN RESISTANCE:

Insulin resistance refers to the decreased rate of glucose uptake mediated by insulin. It was shown to be accompanied by increased levels of insulin and undesirable changes in cardiovascular risk factors like high level of triglycerides, decreased HDL cholesterol, and development of hypertension. Cross-sectional studies have reported that insulin resistance is associated with atherosclerosis which is ultrasonographically viewed in the absence of dyslipidemia and hypertension. It has been proposed that 11β hydroxysteroid dehydrogenase present in omental adipose tissue generates cortisol and causes cushingoid distribution of fat under the influence of hypeinsulinemia. IN addition, adipose tissue is now recognized to be a source of a number of inflammatory cytokines (interleukin – 6) , tumour nerosis factor α(TNF α), growth factors (heparin binding epidermal growth factor (HB-EGF) hormone like substances (leptin, adiponectin, resistin).[50]

HYPER TRIGLYCERIDEMIA:

Hypertriglyceridemic state promotes the oxidative and proinflammatory milieu enhancing expression of adhesion molecule formation of foam cell and intoxication of smooth muscle cell . Following hydrolysis, chylomicrons which are exogenously derived , VLDL cholesterol secreted endogenously enriched remnant by products enters the endothelial space. Hypertriglyceridemia increases reverse cholesterol transport It has been reported that 10% lowering of TGL concentration decreases the risk of CHD by 23%.[46]

HYPERTENSION:

Cardiovascular risk increases both with increasing systolic and diastolic pressure. Plasma cholesterol is a continuous variable like blood pressure and there is no comprehensible cut-off value, but hypertension doubles the risk of CHD at any given concentration of cholesterol. Because intrasubject blood pressure measurements can vary so much over time , the diagnosis of hypertension relies on the measurement of blood pressure on several occasions. Blood pressure increases with age and its prevalence varies with ethnicity. Moreover hypertension is associated with obesity and dyslipidaemia contributing to the augmented risk of CVD. [51]

HYPERHOMOCYSTEINAEMIA:

Homocysteine , a sulphur containing aminoacid, derived from methionine, the concentration of which is modulated by dietary vitamin B6, B12 and folate. The normal plasma homocysteine concentration is 5 - 15µmol/L. Very high concentration of homocysteine (> 100 µmol/L) is toxic to endothelial cells and contributes to premature vascular disease due to free radical mediated mechanism. High concentration of homocysteine inhibits Nitric Oxide (NO) indirectly by stimulating superoxide anion production from endothelial cells. Furthermore it increases the atherogenecity of Lp(a) by liberating free apo(a) impeding fibrinolysis. There is a dose dependent increase in cardiovascular risk with rising plasma homocysteine concentration, synergistic with other coronary risk factors and a number of established coronary risk factors are associated wth raised concentrations of homocysteine, including smoking and renal impairment.[50]

SMOKING:

Cigarette smoking was a well known independent predictor of fresh coronary lesion .An alteration in NO biosynthesis accounts for both primary and secondary effects on the initiation and progression of atherosclerosis. The well known risk factors such as Smoking, poor diet, and lack of exercise contributes to the occurrence of coronary heart disease.[52] Several studies have shown that

Cigarette Smoking causes about 20% to 25% raise in the leukocyte count in the peripheral smear.[53]

The increased susceptibility for smoking associated atherosclerosis and multi vessel CAD is due to CYP1A1 MSP polymorphism and certain endothelial NO synthase intron 4 polymorphisms.

Smoking cessation is effectual in the secondary prevention of coronary heart disease. A recent systematic review have shown that continuous 3 to 7 years after smoking cessation in persons with known coronary heart disease was 30% decrease in crude risk rate of death and myocardial infarction (MI). [54]

LOW PHYSICAL ACTIVITY :

Sedentary life style with reduced physical activity leads to an early development of CHD. Regular physical exercises increase the concentration of HDL and decrease both body weight and blood pressure which are beneficial to cardiovascular health. Recent data indicate that approximately 30min/day of moderate exercise is required to have a significant impact on coronary risk. Exercise –based rehabilitation for patients with CHD is effective in reducing total mortality and lipid concentration.[55]

OBESITY:

Obesity is a well known risk factor for CHD and also growing in prevalence throughout developed and developing countries world wide. Body mass is positively related to fasting triglycerides concentrations, plasma cholesterol and blood pressure, and inversely related to HDL- cholesterol. It is the central or visceral obesity , measured as waist circumference , that is most strongly related to insulin resistance and CHD risk. Waist circumference is a significantly better index of insulin resistance than either waist/hip ratio or BMI. A cut off value for waist circumference of <100 cm rules out insulin resistance in both male and female with optimal sensitivity and specificity. Greater the weight gain more is the risk of hyper tension, CHD and insulin resistance diabetes mellitus . Body mass index of > 30 Kg/m2 is considered as "obesity" and it plays a major role in atherosclerotic progression.[56,57]

ALCOHOL INTAKE:

Studies have proved that consumption of more than 89 g/day of alcohol showed high prevalence of CVD.[58] Several population studies have reported that the relationship between ethanol consumption and CHD risk is J-shaped , with a nadir for risk at approximately three units(30mL) of ethanol per day. Alcohol consumption increases the plasma lipid parameters like triglycerides, VLDL and the large, buoyant LDL subfraction , rather than small, dense LDL which contributes to a proatheroenic state.

INFLAMMATION AND INFECTION:

Atherosclerosis involves many mechanisms similar to a chronic inflammatory disease, the course of its evolution being characterised by T lymphocyte and macrophage permeation. The potential stimuli to inflammatory process include homocysteine, oxidized LDL , infectious micro-organisms and the free radicals generated from cigarette smoking. In the absence of neutralisation of these insults, persisting inflammation results, which might cause local and systemic release of cytokines and growth factors. This is followed by thickening of the intimal layer due to stimulation of extracellular matrix elaboration and smooth muscle cell migration and proliferation. The activated leucocytes release IL- 1β and IL-6 which leads to increase in hepatic CRP synthesis.

Over the history of few years, there exists an increasing interest of utilization of markers of inflammation to predict the events of acute coronary disease. To some extent the predictive value of these markers perhaps related to their capability in recognizing patients with vulnerable plaques rich in activated leukocytes. It has been found that high concentration of CRP in plasma is a stronger risk factor than elevated von Willebrand factor or Erythrocyte Sedimentation Rate (ESR) but has been found to be weaker than passive smoking or elevated cholesterol.

It has been found recently that CRP is deposited in those regions of human coronary vessels which are rich in lipid. CRP has the ability to induce

chemotaxis in monocyte. Hence it is followed by recruitment of monocytes. CRP also activates complement. There is a relationship between the levels of CRP and basal NO synthesis by endothelial cells. All this suggests that CRP may be involved in the early phases of atherosclerosis pathogenesis.

The role played by infectious micro organisms in the development of atherosclerosis is debatable. Over the last few decades, micro organisms like Helicobacter pylori and Chlamydophila pneumonia have been implicated in the pathogenesis of artherosclerosis and it has been postulated that treatment of patients with antibiotics may decrease the risk of artherosclerosis. These micro-organisms have been found in the plaques in human beings and those with acute coronary syndrome have been found to have higher titers of antibodies to these organisms.

Autoimmune mechanism has also been implicated in the pathogenesis of atherosclerosis. Some micro-organisms elaborate proteins called chaperonins or heat shock proteins – 60 (Hsp 60). These are homologous to a protein synthesised in the vascular endothelium. It is postulated that an immune response mounted against this protein may react with endothelial heat shock proteins resulting in injury to the endothelium. Autoimmunity has been postulated as the link between atherosclerosis, endothelial dysfunction and infection. Chlamydia, Cytomegalovirus and Herpes are some of the infectious agents suspected to contribute to atherosclerosis.[59]

ATHEROSCLEROSIS :

DEFINITION

Atherosclerosis is a chronic, degenerative, inflammatory condition affecting medium sized and large arteries coupled with lipid deposition and matrix proteins in the arterial wall, ultimately causing narrowing of the lumen of the arteries.

An atheromatous plaque is characterised by a raised lesion with a soft, yellow grumous core of lipid (chiefly cholesterol and cholesterol esters) covered by a firm, white fibrous cap. Atherosclerotic plaques deteriorate the underlying media beside obstructing blood flow, and can also undergo rupture resulting in acute thrombosis.[59,60]

PATHOPHYSIOLOGY:

The origin and progression of atherosclerosis were explained by several hypothesis which are not mutually exclusive. Moreover many experimental and clinical observations are shown how processes explained in one theory are linked to those in another.

- THE LIPID OXIDATION HYPOTHESIS
- MONOCLONAL HYPOTHESIS
- REACTION TO INJURY HYPOTHESIS
- ENCRUSTATION HYPOTHESIS
- INSUDATION HYPOTHESIS:

INSUDATION HYPOTHESIS:

The significant events of atherosclerosis points to focal accumulation of fat inside the vessel wall which explains the presence of lipid derived from plasma lipoproteins dependable with the role of serum lipids as risk factor for myocardial infarction. This hypothesis not completely explains the pathogenesis since lipid deposition though it is necessary for plaque formation but not sufficient to explain atherosclerosis.

Low density lipoprotein (LDL) are too large (20 nm diameter) to penetrate the tightly closed endothelial junctions. However , transport transversely into an intact endothelium both by receptor- mediated uptake of lipoprotein and by nonspecific uptake in to micropinocytic channels are possible .Lipid may be engulfed by macrophages and transported into the vascular wall and transmigrate between dysfunctional endothelium.

ENCRUSTATION HYPOTHESIS:

This hypothesis explains the deposition of blood materials such as fat, smooth muscle cells and Extra Cellular Matrix (ECM) on the inner surface of the arteries leading to the inner wall thickening. Experimental studies of hyperlipidemic animals and autopsy studies of children show that mural thrombi do not initiate atherogenesis but it is a critical part of delayed evolution of atherosclerotic lesions which is the foremost event leading to vascular occlusion particularly in coronary arteries.

REACTION TO INJURY HYPOTHESIS:

This hypothesis suggests that the proliferation of smooth muscle depends on discharge of polypeptide growth factors secreted by endothelial cells (ECs), macrophages and smooth muscles which accumulate at the injury site. It also explains the cellular responses that occur during atherosclerogenesis constitute fibroproliferative and inflammatory response to injury which led to the discovery that platelet derived polypeptides eg: fibroblast growth factor , transforming growth factor –β ,thrombin, LDL, endothelin, etc., that are required for growth of smooth muscle.

MONOCLONAL HYPOTHESIS:

Studies have established that many plaques are monoclonal, originating from one or very few smooth muscles. The monoclonality of the fibrous cap indicates that some indefinite etiologic factor may induce the formation of fibrous cap by changing growth control of smooth muscle cell in the arterial wall.

THE LIPID OXIDATION HYPOTHESIS:

Nitric oxide decay is accelerated by increased production of reactive oxygen species, the production of which is augmented by prolonged hyperlipidemia, predominantly hypercholesterolemia . Macrophages of ECs produces oxygen free radical which induces the accumulation of oxidised lipoproteins and it take up the oxidised LDL particles via the scavenger receptor leading to the formation of foam cells. Ultimately , oxidized LDL is cytotoxic to ECs and

SMCs leading to EC dysfunction through stimulation of liberation of growth factors, cytokines and chemokines by ECs and macrophages which in turn enhances the recruitment of monocytes into the lesions.

STAGES OF ATHEROGENESIS:

Stage1: Chronic endothelial injury

Stage 2: Accumulation of lipoproteins

Stage 3: Resultant inflammation and factor release

Stage 4: Recruitment of Smooth muscle cell followed by proliferation and ECM production

THE ROLE OF CHRONIC ENDOTHELIAL INJURY:

The inflammatory processes mediating the endothelial dysfunction is usually inactivated by the laminar pattern of blood flow in the lesion protected areas of the vessel wall. Hemodynamic disturbances like low shear stress and areas of turbulent flow make the ECs liable for atherosclerosis. Chronic endothelial injuries which lead to endothelial dysfunction are usually unnoticed and starts from first decade of life. This leads to increase in permeability, adhesion of leukocyte and high thrombotic potential. Accumulation of lipoproteins mainly LDL, with its high cholesterol content in the vessel wall occurs. Lesional lipoproteins are modified by oxidation of the amino group of the lipoprotein leading to the formation of aldehydes. Migration of monocytes

into the intimal layer and transformation into macrophages and lipid filled foam cell follow the adhesion of monocytes to the endothelium .This eventually results in the platelet adhesion, discharge of factors from macrophages, activated platelets and vascular cells leading to the migration of smooth muscle cells into intima from media and smooth muscle proliferation and amplification of extracellular matrix causing accumulation of collagen as well as proteoglycan.[62,63]

ROLE OF INFLAMMATION:

Adhesion of leukocytes is not supported by the endothelium of normal blood vessels. Expression of selective adhesion molecules(IL-1 , TNF) in the presence of atherogenesis, helps in leukocyte binding. Macrophages also generates many cytokines like monocyte chemotactic protein – (MCP - I) which recruits many leukocytes in the plaque.

ROLE OF LIPIDS:

Mutations yielding defective apoliproteins causes dyslipidemia predisposing to the formation of atheromatous plaque. Furthermore, conditions like Nephrotic Syndrome, Alcoholism , Hypothyroidism and Diabetes Mellitus also causes dyslipidemic state which inturn leads to the formation of atherosclerotic plaque.

ROLE OF SMOOTH MUSCLES:

Smooth muscle cells migrate from media in to intima undergoes proliferation and deposits ECM components changing a fatty streak in to a mature fibrofatty

atheroma. ECM growth factors like PDGF, FGF, TGF – α helps in this process.

HIGH RISK PLAQUES:

They have

a. An active inflammatory environment – intrinsic and systemic
b. A thin fibrous cap with a large lipid core filled with procoagulant substances
c. Endothelial denudation and fissuring caused due to release of metalloproteinases
d. Local high shear stress, usually at branching points

COMPLICATED ARTHEROSCLEROTIC PLAQUES:

Progression of a simple fibrofatty artherosclerotic plaque to a complicated one can occur due to:

a. Calcification occurring in necrotic areas and elsewhere in the plaque synchronised by osteoblast and osteoclast like cells present in the vessel wall,
b. Mural thrombosis resulting from turbulent flow around the plaque causing damage to the endothelial lining making it non thromboresistant
c. Structural and functional changes making it vulnerable to destabilisation and

d. Destabilisation of an atheroma, often leading to acute coronary syndromes, occur possibly due to mural thrombus or fibrous cap rupture or hemorrhage inside the plaque. [61]

COMPLICATIONS DUE TO ATHEROSCLEROSIS:

The size and location of the affected vessel and the chronicity of the process are the determinents of further complications.

a. **Sudden occlusion**: Abrupt occlusion of the myocardial arteries may occur due to the thrombosis resulting in ischemic necrosis of the tissue supplied by the vessel.
b. **Chronic narrowing of the vessel lumen**: Growth of the plaque impinges on the lumen leading to progressive reduction in blood flow to the tissue causing chronic ischemia.
c. **Aneurysm formation**: Extension of the complicated lesion of artherosclerosis into the media of an elastic artery proceed to the weakening of the wall ultimately ending in the formation of aneurysm.
d. **Embolism**: Detachment or dislodgement of thrombus produced over an atherosclerotic plaque.

If the rupture leads to total thrombotic occlusion in the coronary vessels, the event is usually an STE AMI. If lesser degrees of occlusion occur, an NSTE AMI or UA may ensue.

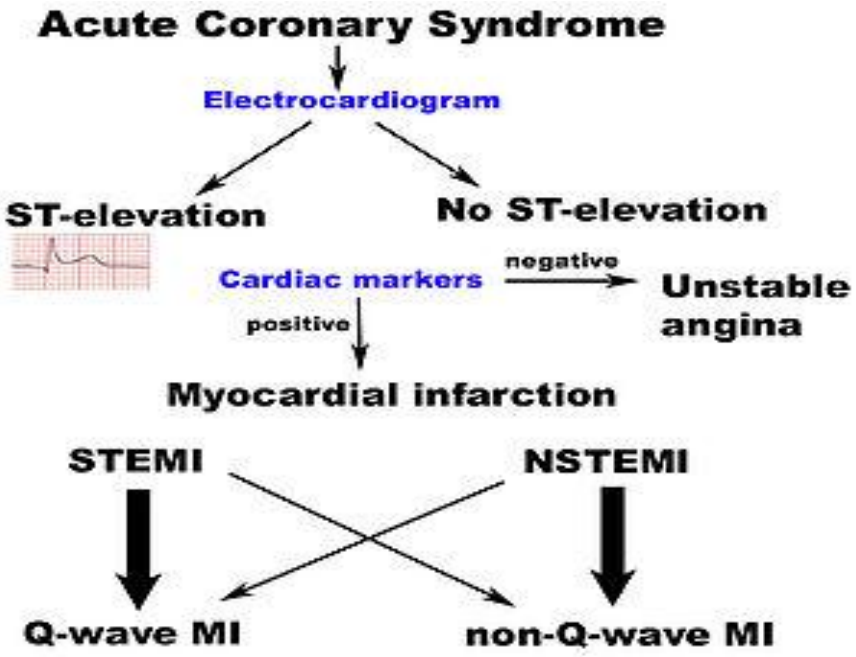
SYMPTOMS OF ACS:

- Severe chest pain at sternum, mostly radiates to the jaw and to the shoulders to one or both arms
- Tightness around the chest radiating to the left arm and to the left angle of the jaw.
- Nausea , vomiting
- Diaphoresis or sweating
- Dyspnoea or Shortness of breath.
- Atypical chest pain (experienced in different ways or even being completely absent which is more likely in diabetic female patients.
- Anxiety
- palpitations
- Angor animi (sense of impending doom)
- Feeling acutely ill.[64]

ECG ABNORMALITIES:

ECG changes occur due to inability of cells affected by ischemia and necrosis to produce normal electrical activity. Various ECG changes like conduction abnormalities, arrhythmias and ST- T abnormalities may occur. ST-T abnormalities may range from peaking of T waves in the hyperacute phase , elevation of ST segment also known as injury current , inversion of T wave and ST segment depression . Damage to the specialised conduction tissues like sinus

node, AV node and other tissues may result in conduction disturbances. While most of these changes are temporary, some may become permanent.[65]



DIAGNOSIS OF AMI:

1. World Health Organisation (WHO) criteria for an acute myocardial infarction (1986): Presence of atleast two of the following

   a) Complaints suggestive of coronary ischemia for a prolonged period.(> 30 minutes)

b) Evolutionary changes on sequential ECG indicative of myocardial infarction.

c) An increase or decrease in serum cardiac markers steady with myocardial ischemia or myocardial necrosis.

2. Criteria for the Definition of Acute Myocardial Infarction (European Society of Cardiology/American College of Cardiology (2000 updated in 2007 (Global task force))

a. Detection of rise and / or fall of cardiac biomarkers (preferably troponin) above 99 th percentile of the upper reference limit along with evidence of ischemia in addition to atleast one of the following:

i. Symptoms suggestive of ischemia

ii. ECG changes of fresh ischemia (new ST-T changes or new Left Bundle Branch Block (LBBB)

iii. Evidence of pathological Q waves on ECG

iv. Imaging based evidence of recent loss of viable myocardium or latest regional wall motion abnormality

4. Diagnosis of Established Myocardial infarction: Anyone of the following criteria satisfies the diagnosis of established MI

a. Appearance of new pathologic Q waves on consecutive ECGs. The patient may or may not recollect the symptoms. Normalised biochemical markers of myocardial necrosis depending on the length of time that has passed.
b. Pathologic findings of a healed or healing MI.

Apart from clinical and ECG findings cardiac biomarkers have become indispensable in the diagnosis of AMI.

CARDIAC BIOMARKERS.

The term ‘biomarker’ , an abbreviation for “biological marker” is defined as a trait that can be objectively measured and evaluated as an indicator of normal biological processes , an important component or event of the pathogenic processes or pharmacological responses to therapeutic interventions.

FDA Definition of Biomarker:

“Any measurable diagnostic indicator that is used to assess the risk or presence of disease.”

Morrow and delomos criteria for biomarkers:

1. Accurate repeated measurements at reasonable cost
2. Must provide additional information.
3. Should aid treatment.

The History of Cardiac Biomarkers

The first cardiac biomarker which was found to be elevated was Aspartate transaminase (AST) in 1954 and used in clinical practice those days. Lack of specificity and the presence of Aspartate transaminase in heart, liver, skeletal muscle, kidneys and brain directed its use currently as a clinical marker for liver damage**.**

In 1959, plasma creatine kinase(CK), was found to be elevated in response to myocardial damage, which catalyses the shift of high energy bond from creatine phosphate to adenosine triphosphate but it demonstrated high sensitive index for skeletal muscle.

In 1960, lactate dehydrogenase (LDH) came into play and it catalyses the reversible oxidation of lactate to pyruvate. However, LDH is found in all cells and like AST, is very nonspecific. CK was considered to be more specific than either LDH or AST because those with dysfunction in hepatic system low levels of CK in the liver are less confounding. [66]

In 1979, despite of this specificity , especially in patients having muscle and hepatic dysfunction WHO had recommended CK, AST and LDH as the biomarker components for diagnosis of AMI.

Identification and categorisation of isoenzyme of both CK and LDH were made available through electrophoresis. CKMB have four fractions of isoenzymes of which CKMB levels were found to be high in cardiac muscle (25–30%) against skeletal muscle which was only 1% comprising of mostly CKMM. Later on, CKMB, CKMB fraction or CKMB/CKMM ratio measurement appeared to be more specific marker for AMI.

Like CKMB LDH also have four isoenzymes of which cardiac muscle is rich in LDH 1 (HHHH) and LDH 2 (HHHM) skeletal muscles contain contains primarily LDH 4 and 5. Presence of more H units represents well oxygenated state while during the injury phase there is a reduction in ratios of H units. Although the measurement of both CKMB and LDH lack specificity lacking in specificity**,** a revolution in the detection and estimation was made by the emergence of immunoassays incorporated into technical advanced automation using polyclonal antibodies and with monoclonal antibodies in 1980s.Monoclonal

antibodies are used in the measurement of CKMB mass which enabled rapid and earlier detection of myocardial damage and was also more sensitive and specific than the original CKMB activity assay. However, skeletal muscle injury , non-ischaemic cardiac disease and some malignancies also showed increase in CKMB mass.

Since CKMB was non- specific , tests with better performance were sought for. The identification of troponin was a crucial advancement in this scenario. Though cardiac as well as skeletal musculature harbour troponin, cardiac isotypes like cTnI and cTnt differ from those in the skeletal muscle owing to the presence of additional aminoacids on the N-amino terminal which makes them easily identifiable. In 1970s, Troponin was discovered to be a constituent of muscle myofibril. Only in the late 1980s, efficient radioimmunoassays became available for cardiac troponin. Though the use of CKMB as a marker of myocardial necrosis had aleady been proposed its superiority to CK and CKMB had not been elucidated. In 2000, cTn was declared to be the preferred biomarker in the guidelines for AMI diagnosis. Initially there was a increase in the 'positive' rate, but the assay was not standardised and the association between histopathology and cTn was not clearly elucidated. As a result it was met with scepticism. But it eventually turned to acceptance in a widespread manner. There also arose an acknowledgment of the variablity in the assay. Therefore cut off values with coefficient of variation of <10% were recommended to be employed. Now recommendations suggest 99^{th} percentile which is lesser than the levels

previously used in practice. But guidelines for such a precision were unachievable by many present assays.

CLASSIFICATION OF BIOMARKERS IN ACUTE CORONARY SYNDROME:[68]

ESTABLISHED MARKERS

1. Troponin I
2. Troponin T
3. (BNP)Brain Natriuretic Peptide
4. N – Terminal Pro – BNP
5. C-Reactive protein
6. Cystatin C
7. Heart – Fatty acid Binding Protein.

EMERGING MARKERS:

1. Myeloperoxidase
2. Metalloproteinase
3. Soluble CD40 ligand
4. Ischemia Modified Albumin
5. Pregnancy associated plasma protein-A

BIOMARKERS UNDERSCORING DIFFERENT FACETS OF THE PATHOPHYSIOLOGICAL AND OUTCOMES OF ACS

1. INFLAMMATION

- C- Reactive protein
- Myeloperoxidase
- Matrix metalloproteinase
- Soluble CD40 ligand

2. PLATELET ACTIVATION

- Soluble CD40 ligand

3. VULNERABLE PLAQUE

- Pregnancy – associated plasma protein-A
- Myeloperoxidase
- Placental growth factor
- Matrix metalloproteinase

4. MYOCARDIAL NECROSIS

- Creatine phosphokinase and isoenzymes
- Troponin I and T
- Fatty acid binding protein

5. ISCHAEMIA

- Ischaemia Modified Albumin

6. PUMP FAILURE

- Brain Natriuretic peptide
- NT- pro Brain Natriuretic peptide

CARDIAC TROPONINS:

Cardiac troponins T and I are the principal cardiac regulatory proteins that control the calcium mediated interaction between actin and myosin. Troponins are released from myocytes when irreversible damage occurs. It is highly specific to cardiac tissue and almost accurately diagnoses myocardial infarction with a history of ischaemc pain or ECG changed reflecting ischaemia. Serum cTnI and cTnT estimations are better expressing more sensitivity and specificity to cardiac musculature and in the recognition of damage in the myocardium. Monoclonal antibodies against the epitopes of both are used in the measurement of cardiac troponins. [70]

CREATINE PHOSPHATE – MB:

Creatine Kinase (CK) which is a muscle enzyme exists in three isoenzymes. CK-MM (skeletal muscle), CK-MB(myocardium) and CK-BB (brain). Around 90% of CK-MB are present in the myocardium but trace amounts are found in small intestine , tongue, diaphragm and uterus . These isoenzymes are present in the cytosol and aid in the transfer of high energy phosphates into and out of mitochondria , thus helps in regeneration of ATP in the cells. In human

myocardial biopsy material, concentrations of CK-MB have been reported to be 100 fold greater in patients with aortic stenosis, coronary artery disease etc. The pathophysiological basis for increase in CK-MB is by creating a state of myocardial hypoxia which causes heart to respond to increased workload by increasing its mass and thus leading to its elevation.[71]

MYELOPEROXIDASE (MPO):

Inflammatory cells like activated neutrophils and monocytes inside the atherosclerotic plaque produces MPO which acts as a mediator enzyme. MPO is known to induce oxidative damage of tissues by generating reactive oxidised intermediates . Low density lipoprotein (LDL) isolated present inside the atherosclerotic lesions found to have increased levels of such intermediates. MPO expression levels are reported at sites of rupture of the plaque , superficial erosions and in the lipid core in patients followed with sudden death. Whereas little MPO expression were found fatty streaks of less harm. [72]

C – REACTIVE PROTEIN:

CRP is an acute phase reactant produced in response to interleukin (IL-6)in the liver . It is very stable pentamieric protein and its concentration in the serum can increase many folds during the acute phase response. In January 2003, CRP was recommended as an inflammatory marker for recognition of cardiovascular risk by both the Centre for disease Control and Prevention (CDC) and the American Heart Association (AHA). The high sensitive CRP assay (hs-CRP) was developed originally to aid evaluation of conditions

associated with inflammation in otherwise healthy patients. Identification and stratification of patients used Cardiac CRP (cCRP) assays to assess the future CVD associated events. [73]

SOLUBLE CD40 LIGAND:

sCD40 ligand is one of the signalling protein points to both inflammatory and platelet interaction. Increased levels of sCD40L have reported to be correlated with an increased risk of cardiac events and it is expressed by activated platelets and increased plasma levels have been associated with platelet activation. [74]

PREGNANCY ASSOCIATED PLASMA PROTEIN-A:

PAPP-A is a high molecular weight (- 200 kDa) glycoprotein synthesized by the syncytiotrophoblast , a zinc binding metalloproteinase classified under matrix metalloproteinase (MMP) which was formerly identified in the plasma of pregnant women. Elevated levels of PAPP-A were found in patients presenting with unstable plaque. It is usually estimated during pregnancy in case of screening for Down Syndrome. Human fibroblasts contains PAPP-A and released into circulation during the disruption of atherosclerotic plaque. Bayes-Genes et all demonstrated an increased PAPP-A concentrations in serum of patients with both unstable and acute coronary syndromes. [75]

PAPP-A has also been evaluated as a marker of cardiovascular risk in symptomatic hyperlipidemic individuals showing a correlation with the degree of echogenicity of carotid atherosclerotic plaques.

In a preliminary study, PAPP-A showed patterns are highly variable release ranging from 2 to 30 hours after the onset of chest pain.

HEART FATTY ACID BINDING PROTEIN:

H-FABP is a low molecular weight protein (14-15)kDa which is very stable and abundant made of -132 amino acids . It is a soluble cytoplasmic protein of myocardial cells. It is involved in the transport and metabolism of fatty acid and expressed mainly in the myocardium. However , brain, kidney and skeletal muscle also have shown its minimal expression. Small size and water solubility facilitates rapid diffusion through the interstitial space and appears as eary as 90 minutes after symptom onset and peaks within 6hours due to rapid renal clearance. [75]

PLACENTAL GROWTH FACTOR (PIGF).

It is a homodimeric glycoprotein, 46-56kDa in size, belongs to the vascular endothelial growth factor (VEGF) sub family. It exists in two isoforms PIGF-1 and 2 . PIGF -2 has heparin binding domain and is a potent angiogenic factor. In patients with ACS, increased plasma levels of PIGF are associated with adverse cardiac outcomes during long-term follow up. This was emphasised as a

marker of vascular inflammation for risk stratification in patients with ACS rather than general markers of inflammation such as hs-CRP. [75]

BRAIN NATRIURETIC PEPTIDE:

It is a 32 amino acid polypeptide secreted by the ventricles of the heart in response to excessive stretching of heart muscle cells. The release of BNP is modulated by calcium ions. The physiological actions includes decreasing systemic vascular resistance and central venous pressure as well as increasing natriuresis. BNP is secreted along with a 76 amino acid N-terminal fragment that is biologically inactive. [75]

ALBUMIN

Albumin is a nonglycosylated low molecular weight globular protein of 585 amino acids. It has a molecular weight of 66,438 Daltons. 60% of albumin is found in the extravascular space. HSA (Human Serum Albumin) is the most predominant protein in blood with multiple functions. It is produced in the liver. It has a half life of 19 days. Analysed by X ray crystallography, it is found to be folded into three domains which are homologous. Its three dimensional structure has a heart shape which is stabilised by 17 intra chain disulfide bonds. The mean concentration of HSA is 0.63 mmol/L (40g/L). Albumin Cobalt Binding (ACB) test is a recently reported biochemical test exploiting the property of normal serum albumin to bind transition metal cobalt. It was found

that albumin in the serum of patients with myocardial infarction showed reduced binding to cobalt. [76]

PROPERTIES:

- Albumin is relatively stable resisting denaturation than most of the plasma proteins.
- Normal half life of albumin ranges from 15 – 19 days.
- At pH of 8.6 albumin carries a net charge of about – 25 which accounts for its mobility towards anode.
- One unpaired cysteine in 34^{th} position occurs partially in exchangeable disulfide bonds with small compounds such as cysteine and homocysteine.

The metal binding property of HSA has been vastly researched on. It has been found that metallic ions bind to different sites on HSA. Extensive studies of HSA have revealed multiple metal binding properties and the metal ions bind to a extensive range of sites. The binding comprising of the amino acid sequence N-Asp-Ala-His-Lys is very tyical. First three residues have been shown to be necessary for metal ion binding whereas the fourth residue (lysine) not. These sites have high affinity particularly for copper and nickel. The α-amino group, the δ-imidazole nitrogen from His3, the two intervening peptide nitrogen atoms, and the side-chain carboxyl group of Asp1 are specifically involved in this binding property.

BINDING SITES:-

- Site I binds salicylates, Sulfanomides
- Site II – Tryptophan, thyroxine, octanoate
- Imidizole of His 3- forms a binding site for cu++ ions

N terminal- binds metal ions like cobalt, nickel, copper

METABOLISM:

Albumin is synthesized by the hepatic parenchymal cells except in fetal life, where yolk sac is the primary site. Catabolism occurs by pinocytosis by all tissue and the resulting free amino acids areutilized for synthesis of cellular proteins. Small amounts are also lost into the gastro intestinal tract and the glomerular filtrate

FUNCTIONS OF ALBUMIN:

1. Serves as a major component of colloid osmotic pressure inside the blood vessels.
2. Transports variety of substances including fatty acids , bilirubin , drugs calcium , metals etc...
3. Serves as a storage form of amino acids which can be utilised in catabolic states.
4. Increases capillary permeability to small proteins by binding to endothelial membrane associated glycoprotein.
5. Albumin reduces the inflammatory response of platelets and neutrophils by inhibiting leukotrienes .

6. It is essential for the metabolism and detoxification of many compounds.

7. Serves as an important component of protein buffer

DECREASED PLASMA LEVELS:-

1) Due to reduced synthesis as in liver disease .

2) Increased loss

- in urine in nephrotic syndrome.
- Into intestine in protein losing enteropathy.
- From the skin in burns.
- In severe hemorrhage.

3. Impaired uptake in malnutrition.

4. Defective digestion or malabsorption.

ANALBUMINEMIA:-

It's an inherited condition of plasma albumin with levels less than 0.5g/L43.

REFERENCE LEVELS:-

35 to 52 g/l or 3.5 to 5.2 g/dl.

ISCHEMIA MODIFIED ALBUMIN:

N-terminus part of albumin which is either damaged or replaced by copper ion is termed as ischemia modified albumin (IMA) . Conditions necessary for altering the metal binding site of HSA are known to occur in vivo and probably occur within minutes after the onset of myocardial ischemia. [77]

As a result of hypoxia, acidosis, free-radical injury and energy- dependent membrane disruption which occurs during ischemia, the N-terminus of albumin is transformed reducing its binding capacity for metals. Increasing concentration of IMA caused less cobalt binding therefore more residual unbound cobalt available to complex with a chromogen (dithiothreitol) the color of which can be measured photometrically. This is the basis of the albumin cobalt-binding (ACB) test. An increase in IMA is inversely related to the unbound amount of cobalt, causing an increase in coloured product produced in the test platform. [77]

Saif Anwaruddin et al demonstrated increased the sensitivity for detecting ischemia to 97%.with a negative predictive value of 92%. IMA was highly when combination of IMA–myoglobin–CK-MB–TnI estimations were done, which might improve the usefulness of standard biomarkers of myocardial ischemia. They concluded that the results of IMA testing were additive to those of ECG and useful when used in conjunction with markers of myocardial necrosis.[78]

In another study done by Abdul-Fattah et al they proved that Ischemia modified albumin is a very sensitive marker for the early diagnosis of myocardial ischemia before irreversible change like necrosis occur. [79]

METHODS OF MEASUREMENT:

Albumin Cobalt Binding Test

ELISA technique.

MATERIALS AND METHODS:

The study was conducted in Thanjavur Medical College Hospital. 50 patients with symptoms of acute coronary syndrome presented within 6 hours of onset of pain in the causality with ECG findings correlated and were taken as subjects . 50 age and sex matched controls were taken as control group.

INCLUSION CRITERIA:-

1) Patients admitted with complaint of chest pain within 6 hours of onset.

2) Electro cardio graphic findings showing abnormal ST-T wave changes (ST segment elevation or depression or deep symmetrical T wave inversion).

EXCLUSION CRITERIA:-

1) Presence of renal diseases.
2) Presence of cirrhosis.
3) Presence of stroke, skeletal muscle injury, malignancy, trauma.
4) Critically ill patients.
5) Any infectious diseases.
6) Serum albumin < 2 gms/dl ,
7) Serum creatinine > 3 mgs/dl.

BLOOD COLLECTION:-

Informed consent was obtained for each patient and control prior to the study. 5ml of blood samples were collected by vene puncture under strict aseptic precaution as soon as the subjects got admitted as per the inclusion criteria. The samples were centrifuged and serum separated. One part of the sample was taken and analysis of CK-MB, LDH ,albumin and creatinine were done immediately. Remaining part of the sample was stored for analysis of Ischemia Modified Albumin at - 20º C. 12-14 hours fasting samples were collected from all subjects during their hospital stay and analysis of total cholesterol, triacylglycerol and high density lipoprotein were done.

ANALYSIS OF BLOOD SAMPLES:

The serum collected above was used for the estimation of the following parameters

ESTIMATED PARAMETERS:

1. Ischemia Modified Albumin—Albumin Cobalt Binding test
2. Serum Creatine Kinase - Modified IFCC method
3. Serum Lactate Dehydrogense - UV kinetic method
4. Serum Aspartate Transaminase - Modified IFCC Method.
5. Serum Urea - Urease method
6. Serum Creatinine - Modified Jaffe,s Method
7. Serum Albumin - Bromo Cresol Green colorimetric Method.
8. Serum glucose - Glucose oxidase – Peroxidase Method.

9. Total Cholesterol - Enzymatic cholesterol esterase method

10. Serum Triglycerides - GPO – PAP Method.

11. Serum High Density Lipoprotein - Phosphotungstic acid method.

CALCULATED PARAMETERS:

1. Body Mass Index: (BMI): $\frac{\text{weight in Kg}}{(\text{height in meters})^2}$

2. Very Low Density Lipoprotein = TGL/5

3. Low Density Lipoprotein = T- Chol – (LDL + VLDL)

ESTIMATION OF ISCHEMIA MODIFIED ALBUMIN:

The tests were performed by chemical method using Cobalt chloride (hexa hydrate form) Dithiothreitol and Sodium chloride.

PRINCIPLE :-

Free Cobalt that does not bind to the Ischemia modified albumin in serum gives a brown coloured complex with Chromogen dithiothreitol which is measured spectrophotometrically at 470 nm.Intensity of the colour is directly proportional to Ischemia modified albumin in serum.

REAGENTS :-

Cobalt chloride - 1 gm / litre

Dithiothreitol - 1.5 gm / litre

Sodium chloride - 9.0 gm / litre

All reagents are freshly prepared.

STANDARDIZATION OF THE PROCEDURE:-

Preparation of standards - Cobalt chloride [CoCl2.6H2O] was used for preparation of cobalt standards.

STOCK SOLUTION:- [1 gm % of cobalt]

Dissolve 1.83 gms of cobalt chloride in deionised water and make up to 100ml.

SUBSTOCK:- [10,000 µg/10 ml of cobalt]

100 µl of stock solution is made up to 10ml.

WORKING STANDARDS:-

Working standards of various concentrations were prepared from

the sub stock solution as shown in table.

Working standard Concentration (µg/dl)	**Stock solution (µl)**	**Deionised water (µl)**
10	10	990
40	40	960
70	70	930
100	100	900
130	130	870

Standards with Serial concentration were prepared as follows.

CONC	**STD O.D**	**BLANK**	**STD - BLANK**
10	0.142	0.02	0.125
40	0.345	0.02	0.325
70	0.689	0.02	0.659
100	0.904	0.02	0.874
130	1.151	0.02	1.121

REAGENTS:-

1) **COBALT CHLORIDE :-**

0.183 mgs of Cobalt chloride dissolved in 100ml of deionised water.

2) **DITHIOSTHREITOL [DTT]:-**

1.5 gm of Dithiothreitol dissolved in .1 litre of deionised water.

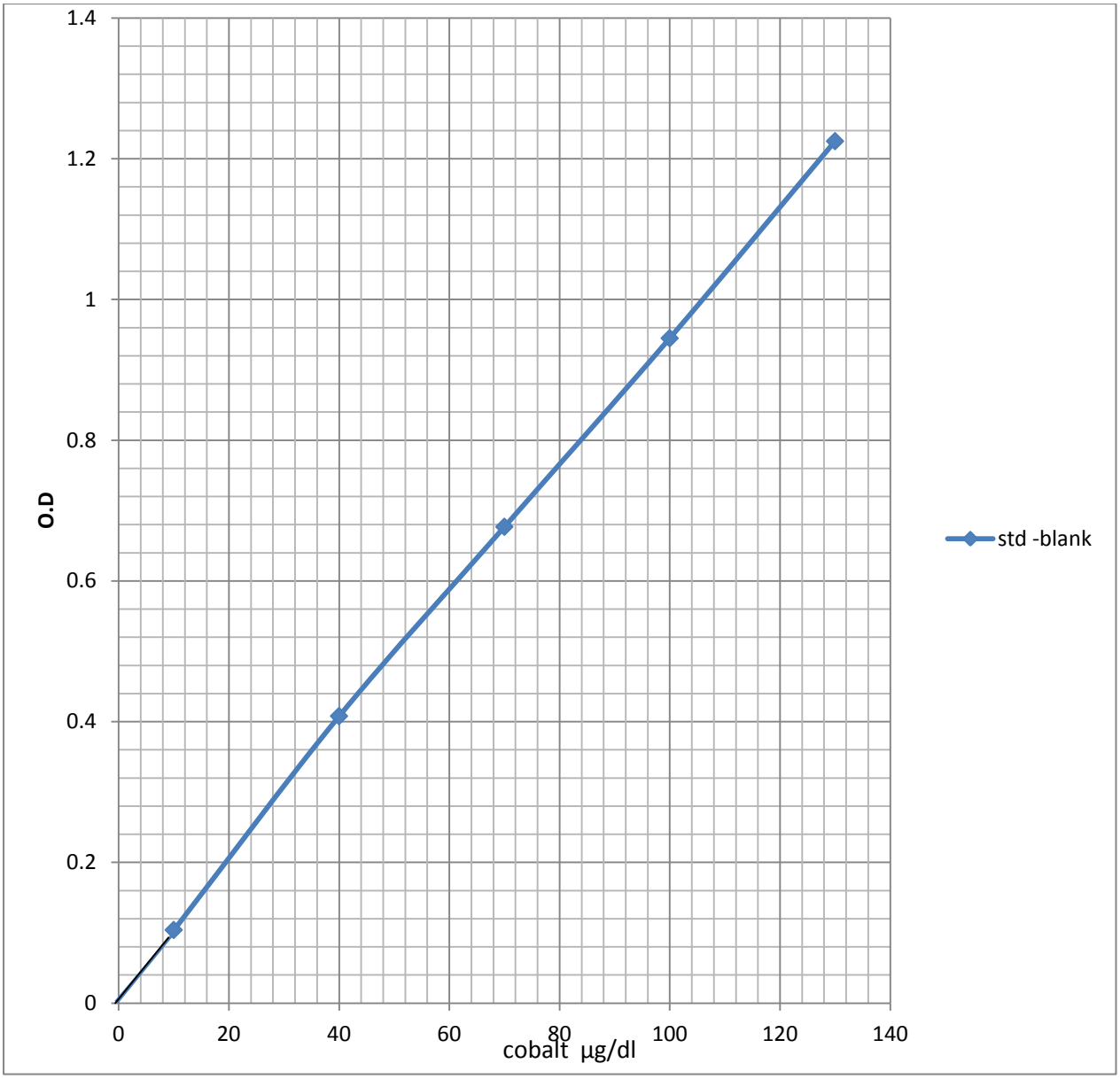
3) 0.9 gms % SODIUM CHLORIDE:-

9.0 grams of Sodium chloride dissolved in 1 litre of deionised water to quench the reaction.

Procedure:

	BLANK	STANDARD	TEST
Sample	--	--	200µl
Cobalt chloride	50 µl	50 µl	50 µl
Deionised water	250 µl	--	--
DTT	--	50 µl	50 µl
NaCl	1ml	1ml	1ml

Add 50µl of cobalt chloride to the serum sample, mix vigorously and after ten minutes of incubation add 50 µl of Dithiothreitol. Wait for 2 minutes and then add 1ml of 0.9 % sodium chloride. Read the absorbance at **470 nm** after 1 minute.



ABSORBANCE OF COBALT STANDARDS:-

Reagent blank = 0.020

CALCULATION:-

Absorbance of unknown x concentration of standard

Absorbance of standard

REFERENCE VALUES:-

6 – 80units/ml.

ESTIMATION OF SERUM (CK-MB)

The tests are performed in reagent kit by **Modified IFCC method.**

PRINCIPLE OF THE METHOD:-

This procedure involves measurement of CK activity in the presence of an antibody to CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB while not affecting the B subunit activity of CK-MB and CK-BB. Then the CK method is used to quantitatively determine CK-B activity. The CK-MB activity is obtained by multiplying the CK-B activity by two.

$$\text{Creatine phosphate + ADP} \xrightarrow{\text{creatine kinase}} \text{Creatine + ATP}$$

$$\text{ATP + D-Glucose} \xrightarrow{\text{hexokinase}} \text{Glucose-6-phosphate + ADP}$$

$$\text{Glucose-6-phosphate + } NADP^{+} \xrightarrow{\text{Glucose 6 Phospate dehydrogenase}} \text{6 - Phospogluconate + } + NADPH + H^{+}$$

REAGENTS:-

REAGENT I:- (Buffer / Enzymes)

REAGENT II:- (Polyclonal Antibody

WORKING REAGENT:-

Add 4ml of reagent I to one ml of reagent II. Mix gently by swirling till completely dissolved.

PROCEDURE:-

The reagent and sample are brought to room temperature

	Blank	Standard	Test
Reagent	1ml	1ml	1ml
Distilled water	50 µL	-	-
Standard	-	50 µL	-
Sample	-	-	50 µL

To 1 ml of working reagent add 50 µl of sample and read immediately at **340** nm

NORMAL VALUES:-

Serum: 0 – 24 u /L.

ESIMATION OF LACTATE DEHYDROGENASE: (LDH)

METHOD: UV - KINETIC METHOD.

PRINCIPLE:

Lactate Dehydrogenase catalyses the oxidation of lactate to pyruvate accompanied by simultaneous reduction of NAD to NADH. LDH activity is proportional to the increase in absorbance due to the reduction of NAD.

$$\text{Lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{pyruvate} + \text{NADH}$$

REAGENTS:

REAGENT 1:

NAD :5mmol/L

Lactate :50 mmol/L

REAGENT 2:

Buffer (pH 9.3 at 25 ºC) 100 mmol/L

WORKING REAGENT PREPARATION: (For 10 x 1.1 ml)

one tablet of 1 LDH was reconstituted with 1.1 ml of 2LDH . Mixed gently to dissolve the contents and used after 5 minutes.

PROCEDURE:

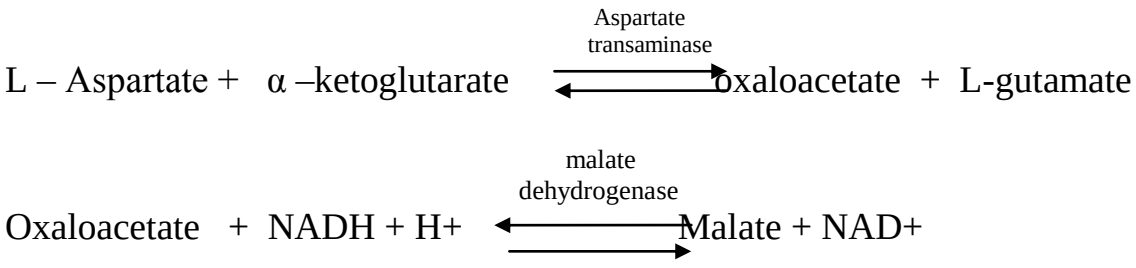
Pipette 1ml of working reagent with 50 µl of sample , mix and read the first absobance at one minute and at 30, 60, 90 seconds at 340nm. The mean change in the absorbance per minute was calculated as enzyme activity.

NORMAL RANGE: 114 - 240 IU/L

ESIMATION OF ASPARTATE TRANSAMINASE: (AST)

METHOD: MODIFIED IFCC METHOD

PRINCIPLE: Aspartate transaminase (AST) catalyses the transfer of amino group from L-aspartate to α –ketoglutarate to yield oxaloacetate and L-glutamate. The oxaloacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase catalysed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity.



REAGENT:1

Tris nuffer (pH 7.8)	20mmol/L
L-Aspartate	230mmol/L
LDH	>33.3µkat/L
2-Oxaloacetate	13.21mmol/L
MDH	>33.3µkat/L

REAGENT: 2

NADH	1.51 mmol/L

REAGENT PREPARATION:

Working reagent was prepared by mixing 4 parts of R1 with 1 part of R2 per assay tube.

PROCEDURE:

500 µl of working reagent was mixed with 25µl of sample , mixed well and aspirated.

CALCULATION:

SGOT (AST) activity (IU/L) = DA/ min x factor (3376)

NORMAL RANGE: 10 – 40 U/L

ESTIMATION OF GLUCOSE:

METHOD: GLUCOSE OXIDASE PEROXIDASE METHOD.

PRINCIPLE:

Glucose in the sample is oxidised to gluconic acid and hydrogen peroxide by glucose oxidase. The enzyme peroxidise catalyses oxidative coupling of 4-aminophenozone with phenol to give pink coloured quinoe -imine complex. The intensity of the colour is proportional to the concentration of glucose present in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + \text{Hydrogen peroxide.}$$

$$H_2O_2 + \text{Phenol} + 4\ \text{AAP} \xrightarrow{\text{peroxidise}} \text{Quinoeimine dye} + H_2O$$

Glucose standard: 100mg/dl

Specimen: fresh unhemolysed serum .

ASSAY PROCEDURE:

Enzyme reagent	1ml	1ml	1ml
Blank	10 µl		
Standard	-	10 µl	-
Test	-	-	10 µl

Mixed well after each addition and incubated at 37ºC for 5 mts. The absorbance of the Standard and the test were read against reagent blank at 505nm.

Calculation:

Glucose (mg/dl) = Absorbance of test × Concentration of standard (mg/dl)
Absorbance of standard

Glucose Standard: 100mg/dl

Linearity: Upto 500mg/dl

Normal Values: Glucose fasting: 65-100mg/dl

Glucose postprandial: 90-140mg/dl

QUANTITATIVE DETERMINATION OF ALBUMIN:-

(BCG DYE BINDING METHOD):-

PRINCIPLE OF THE METHOD:-

Albumin in a buffered solution reacts with the anionic bromo cresol green (BCG) with a dye binding reaction to give a proportionate green colour which is measured at 630 nm (600 – 650 nm).

REAGENTS:-

Reagent I - Bromocresol green

Succinic acid - 94 m mol /L

Sodium hydroxide - 10.2 m mol /L

BCG :- 0.149 m mol /L.

PROCEDURE:-

The samples and the reagent were brought to room temperature before use.

Add 1 ml of reagent to 10 µl of sample, mix incubate for 1 minute at room temperature, and read at 630 nm.

	Blank	Standard	Test
Reagent	1ml	1ml	1ml
Standard	--	10 µL	
Sample	--	--	-- 10 Ml

Standard Concentration: 6gm/dl

Albumin (gm/dl) = Absorbance of test × Concentration of standard (mg/dl)
Absorbance of standard

Normal values = 3.5 – 5 g / dl.

ESTIMATION OF BLOOD UREA:

METHOD: UREASE – GLDH METHOD

PRINCIPLE:

Urea is hydrolyzed to ammonia and carbon dioxide by Urease. Then Glutamate dehydrogenase (GLDH) converts ammonia and α Keto glutarate to glutamate and water with the concurrent oxidation of reduced NADH to NAD^+ . Two moles of NADH are oxidized for each mole of urea present.

$$\text{Urea} + H_2O \xrightarrow{Urease} 2NH_4+ + co_2 .$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2-oxoglutarate} \xrightarrow{GLDH} \text{Glutamate} + NAD^+.$$

The initial rate of decrease in absorbance at 340nm is proportional to the urea concentration in the sample.

Reagent composition:

Reagent 1:

α-Keto glutaric acid 99.8mmol/L

Urease 23.5ku/L, GLDH 3.5ku/L, Adenosine diphosphate 7.6mmol/L, Sodium Acid 0.2%.

Reagent 2: NADH 2.95mmol/L, sodium Acid 0.1% urea standard 50mg/dl.

Reagent Preparation:

Working reagent was prepared by mixing 4 parts of reagent 1 with one part of reagent 2.

Procedure: 3 test tube were labeled as Blank, standard and test and the procedure is done as follows:

Tubes	Working reagent	Standard	Test sample	Distilled water
Blank	1000µl	-	-	10µl
Standard	1000µl	10µl	-	-
Test	1000µl	-	10µl	-

The tubes were mixed well and the absorbance was read after 20 seconds (A_1) and 60 sec (A_2) at 340nm.

Calculation:

$\Delta A = A_2 - A_1$.

$$\text{Urea in mg/dl} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{Concentration of standard (50mg/dl).}$$

Linearity: The method is linear upto 200mg/dl.

Reference Interval: 15-30mg/dl.

ESTIMATION OF SERUM CREATININE

Method: Modified Jaffe's Method.

Principle: Creatinine reacts with alkaline picrate to produce an orange-yellow colour. The intensity of the colour is directly proportional to the concentration of Creatinine and is measured photometrically at 510nm.

Reagent Composition:

Reagent No	Composition	Concentration
1	Picric acid	25.8mmol/L
2	Sodium hydroxide	95mmol/L

Concentration of standard creatinine 2 mg/dl

Reagent Preparation

Equal Volumes of reagent 1 and reagent 2 were mixed and waited for 15 minutes before use.

Procedure: 3 test tubes were taken and labeled as Blank, Standard and test and the procedure was done as follows:

Tubes	Working reagent	Standard	Test sample	Distilled water
Blank	1000µl	-	-	100µl
Standard	1000µl	100µl	-	-
Test	1000µl	-	100µl	-

The tubes were mixed well and the absorbance was read after 20 seconds (A_1) and 80 sec (A_2) at 510nm, against reagent blank with distilled water.

Calculation: $\Delta A = A_2 - A_1$.

Serum Creatinine (mg/dl) = $\frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times$ Concentration of standard

Linearity: This assay is linear upto 20mg/dl.

Reference Range: Males: 0.7-1.4 mg/dl

Females: 0.6-1.2mg/dl.

ESTIMATION OF TOTAL CHOLESTEROL:

Method: Cholestrol Oxidase – PAP method, End Point Analysis.

Principle:

1.Cholestrol Ester + Water $\xrightarrow{Esterase}$ Cholestrol + fatty acid

2.Cholesterol + oxygen $\xrightarrow{Cholesterol\ Oxidase}$ Cholest – 4- en-3 one + H_2O_2

3. $2H_2O_2$ + 4AAP + Phenol $\xrightarrow{Peroxidase}$ Quinoneimine dye + 4 H_2O

4AAP - 4 Amino antipyrine

Absorbance of quinoneimine is directly proportional to cholesterol concentration.

Reagent composition:

Goods buffer (PH 6.4) : 100mmol/L

Cholesterol Oxidase : >100U/L

Cholesterol Esterase : >200U/L

Peroxidase : >3000 U/L

4- Amino anti pyrine : 0.3mmol/L

Phenol : 5mmol/L

Procedure:

Reagents	Blank	Standard	Sample
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

The tube were mixed well and incubated for 10min at room temperature. The absorbance of the test and standard were read against reagent blank at 505nm.

Calculation:

Cholesterol in mg/dl = $\frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times$ Concentration of standard (mg/dl).

Concentration of cholesterol standard 200mg/dl.

Reference Range

AGE	SERUM CHOLESTEROL(mg/dl)
2-12 months	60-190
≥1year	110-230
Adults	<200

Linearity: This method is linear upto 700mg/dl

ESTIMATION OF TRIGLYCERIDES

Method: Enzymatic method with glycerol Phosphate oxidase (GPO – PAP method)

Principle:

$$\text{Triglycerides} + H_2O \xrightarrow{lipase} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[Mg2+]{Glycerol\ kinase} \text{Glycerol 3 Phosphate} + \text{ADP}$$

$$\text{Glycerol 3 Phosphate} + O_2 \xrightarrow{GPO} \text{DAP} + H_2O_2$$

ATP – Adenosine Triphosphate, 4 AAP 4 Amino Antipyrine, DHBS—3,5 Dichloro 2-Hydroxy Benzene Sulfonate

The Intensity of the Quinoneimine dye formed is proportional to the Triglyceride concentration in the sample when measured at 540nm.

Reagent Composition:

Reagent 1 (Enzymes/Chromogen):

Lipoprotein lipase	4000U/L
4-Amino Antipyrine	0.4 mmol/L
ATP	2 mmol/L
Glycerol Kinase	1500U/L
Peroxidase	2200U/L
Glycerol Phosphate Oxidase	4000U/L

Reagent – 2:

Pipes buffer pH7.0: 40mmol/L

DHBS : 0.2 mmol/L

Magnesium salt: 2.5 mmol/L

Working Reagent Preparation:

The working reagent was prepared by mixing 4 parts of R_1 with 1 part of R_2, and is stable for 90 days at 2-8^{0}C.

Procedure:

Three test tubes were taken and labeled as Blank (B), Test (T) and Standard (S). The procedure was as follows:

Reagents	Blank	Standard	Sample
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-		10µl

The tubes were mixed and incubated for 10 minutes. Absorbance was read at 540nm for standard and sample against reagent blank.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard (mg/dl)}$$

Reference Values: 25-160mg/dl

Linearity: Upto 1000mg/dl

HDL CHOLESTEROL ESTIMATION

Method: Phosphotungstic acid / Magnesium Precipitation method.

Principle:

Chycomicrons, VLDL-C and LDL-C are precipitated from the serum by phosphotungstate in the presence of divalent cations such as Magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using cholesterol reagent

$$\text{Serum} \xrightarrow{PRECIPITATION} \underset{\text{(Supernatant)}}{\text{HDL-C}} + \underset{\text{(Precipitate)}}{\{\text{LDL-C+VLDL–C+ Chylomiorons}\}}$$

Reagent Composition:

Precipitating reagent:

Phosphotungstic acid	2.4 mmol/L
Magnesium Chloride	40 mmol/L

HDL Cholesterol standard 25mg/dl.

Precipitation:

Precipitation of LDL-C, VLDL-C and chylomicrons were done by adding 500µl of precipitating reagent into 250µl of serum. The tube was mixed well and allowed to stand for 10 min. Then it was centrifuged at 3000 rpm for 10 min and a clear supernatant was obtained. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

Procedure:

Reagents	Blank	Standard	Sample
Cholesterol working reagent	1000µl	1000µl	1000µl
Distilled water	50µl	-	-
Standard	-	50µl	-
Supernatant	-	-	50µl

Tubes were mixed well and incubated for 10min at room temperature. The absorbance of the standard and the test samples were read at 505nm against reagent blank.

Calculation:

HDL Cholesterol (mg/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$ × concentration of standard ×dilution factor.

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 25 \times 3$$

Linearity: Upto 125mg/dl

Normal Values:Males: 30-65mg/dl

Females: 35-80 mg/dl.

CALCULATED PARAMETERS:-

FRIEDWALD'S FORMULA:

VERY LOW DENSITY LIPOPROTEIN: $\frac{\text{Triacylglycerol}}{5}$

LOW DENSITY LIPOPROTEIN:

Total Cholesterol – HDL – $\frac{\text{Triacylglycerol}}{5}$

MASTER CHART – I (CONTROL GROUP)

S.NO	Age (Yrs)	sex	Wt (Kg)	Ht (mts)	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	IMA (U/dl)	S.ALB (g/dl)	CKMB (U/L)	RBS (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)	T-CHOL (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	LDH (U/L)	SGOT (U/L)
1	54	M	74	1.5	32.88	130	86	24.6	3.5	12	110	25	0.8	146	120	38	84	24	54	21
2	51	M	54	1.58	21.63	136	80	24	3.6	9.45	120	24	0.7	148	141	35	84.8	28.2	85	28
3	54	M	74	1.65	27.1	124	84	22.6	3.8	8.62	110	28	0.9	175	152	34	110.6	30.4	86	24
4	61	M	63	1.58	25.23	136	80	58.75	3.86	14.2	115	22	1.2	146	110	40	84	22	110	25
5	58	M	80	1.59	31.64	138	70	22.6	3.84	12.5	114	24	0.9	165	94	52	94.2	18.8	110	28
6	54	M	62	1.63	23.33	142	74	60	3.8	11.3	117	27	0.6	164	85	42	105	17	121	26
7	49	M	81	1.68	28.69	140	76	52.2	3.9	14.2	102	25	0.8	154	152	40	83.6	30.4	142	23
8	57	M	45	1.45	21.40	140	78	10.6	4.1	12.2	105	24	0.75	158	121	44	89.8	24.2	110	35
9	48	M	49	1.56	20.13	130	70	18.6	3.89	11.3	114	26	0.85	175	142	39	107.6	28.4	151	26
1	49	M	58	1.89	16.23	130	80	12.32	3.6	10	109	25	0.95	148	132	45	76.6	26.4	125	38
11	61	M	85	1.65	31.2	120	82	61.24	3.8	10.2	107	24	0.87	126	123	42	59.4	24.6	132	31
12	60	M	68	1.64	25.28	122	84	57.64	4.2	10.5	116	19	0.86	124	123	32	67.4	24.6	156	35
13	54	M	67	1.54	28.25	132	80	24.32	3.6	11.2	108	26	0.85	142	133	35	80.4	26.6	165	24
14	58	M	81	1.58	32.44	130	80	42.2	4.12	10.6	124	28	0.96	152	142	36	87.6	28.4	164	25
15	52	M	82	1.57	33.26	130	80	24.65	4.3	10.6	113	31	0.76	124	147	42	52.6	29.4	154	26
16	53	M	59	1.68	20.9	120	78	25.46	3.8	12.9	115	21	0.64	152	154	34	87.2	30.8	154	35
17	54	M	69	1.52	29.86	110	70	48.56	3.9	14.2	109	31	0.75	162	121	24	113.8	24.2	168	38
18	58	M	65	1.69	22.75	120	90	52.6	3.8	16.6	122	30	0.65	165	132	45	93.6	26.4	110	24
19	54	M	67	1.64	24.91	120	84	36.98	3.4	14.2	121	19	0.85	168	151	38	99.8	30.2	123	35
20	49	M	68	1.69	23.80	130	80	24.6	3.6	16.2	108	32	0.46	142	141	44	69.8	28.2	123	31
21	51	M	64	1.69	22.40	110	80	28.6	3.54	12.4	105	32	0.65	175	156	41	102.8	31.2	132	21
22	52	M	69	1.66	25.03	120	84	24.26	4.12	1.5	104	32	0.9	157	121	42	90.8	24.2	147	33
23	57	M	68	1.65	24.97	128	82	45.26	4.5	10.3	122	22	0.8	142	128	42	74.4	25.6	154	24
24	55	M	82	1.64	30.4	124	78	54.69	3.8	12.2	123	24	0.6	132	124	47	60.2	24.8	124	26

S.NO	Age (Yrs)	sex	Wt (Kg)	Ht (mts)	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	IMA (U/dl)	S.ALB (g/dl)	CKMB (U/L)	RBS (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)	T-CHOL (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	LDH (U/L)	SGOT (U/L)
25	54	M	80	1.49	36.03	120	80	58.64	3.9	14.2	128	25	0.7	132	134	48	57.2	26.8	110	23
26	49	M	80	1.56	32.87	130	80	44.36	3.6	14.5	114	24	0.68	136	132	39	70.6	26.4	100	33
27	45	M	74	1.49	33.33	130	80	44.26	3.4	13.2	117	21	0.78	163	143	42	92.4	28.6	98	12
28	51	M	75	1.56	30.81	120	80	28.64	3.6	15.2	105	22	0.74	153	152	39	83.6	30.4	85	16
29	48	M	74	1.59	29.27	124	82	26.84	3.5	12.6	115	28	0.97	135	142	35	71.6	28.4	95	25
30	52	M	75	1.69	26.25	126	82	58.64	3.65	16.5	116	26	0.94	163	134	36	100.2	26.8	94	18
31	43	M	74	1.57	30.0	132	82	54.24	3.6	14.3	112	24	0.68	154	125	45	84	25	96	19
32	54	M	76	1.68	26.92	130	80	26.54	3.8	16.2	117	18	0.95	185	124	36	124.2	24.8	98	26
33	52	M	85	1.68	30.11	140	80	25.56	3.7	12.6	98	19	1	13	124	45	-56.8	24.8	84	24
34	51	M	59	1.49	26.57	130	84	42.2	3.4	20.12	94	25	1	135	127	42	67.6	25.4	74	22
35	58	M	68	1.47	31.46	126	82	28.45	3.8	10.2	110	26	0.9	145	126	41	78.8	25.2	56	21
36	46	M	65	1.49	29.27	130	84	45.26	3.9	12.2	95	24	0.75	148	152	40	77.6	30.4	15	27
37	49	F	65	1.59	25.71	130	82	42.8	3.6	10.2	98	22	0.68	147	123	40	82.4	24.6	18	28
38	43	F	69	1.57	27.99	126	80	28.62	3.4	14.6	110	21	0.95	184	142	45	110.6	28.4	99	26
39	45	F	65	1.68	23.03	130	78	26.8	3.6	10.6	112	23	0.74	151	125	46	80	25	110	24
40	48	F	65	1.56	26.7	136	80	48.8	3.8	8.6	120	28	0.78	141	126	48	67.8	25.2	86	22
41	52	F	81	1.68	28.69	126	80	56.6	3.7	13.2	124	24	0.85	154	126	43	85.8	25.2	84	24
42	46	F	80	1.67	28.68	130	70	54.62	4.1	12.8	110	27	0.82	165	128	40	99.4	25.6	79	23
43	49	F	74	1.68	26.21	130	84	19.84	3.6	11	100	19	0.95	156	126	45	85.8	25.2	103	26
44	47	F	75	1.68	26.57	136	80	52.6	3.8	10.8	96	26	0.84	145	124	39	81.2	24.8	98	28
45	45	F	72	1.56	29.5	120	82	26.64	3.6	15.2	95	24	0.85	154	124	38	91.2	24.8	112	24
46	52	F	73	1.69	25.55	110	78	54.2	4.2	12.5	94	31	1	175	125	45	105	25	87	16
47	51	F	82	1.7	28.37	122	70	29.98	4.1	11.8	110	25	0.87	157	145	42	86	29	141	21
48	54	F	71	1.64	26.39	110	80	24.28	4.2	12.2	121	34	0.84	147	168	42	71.4	33.6	68	19
49	51	F	60	1.65	22.03	130	78	24.64	3.6	10.6	110	25	0.85	185	165	41	111	33	92	18
50	56	F	72	1.56	29.5	110	80	48.98	4.1	14.8	106	31	0.98	131	166	40	57.8	33.2	120	25

MASTER CHART-II (STUDY GROUP)

s.no	Age (yrs)	sex	Wt (kg)	Ht (mts)	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	Duration (hours)	IMA (U/dl)	S.ALB (g/dl)	CKMB (U/L)	RBS (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)	T-CHOL (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	LDH (mg/dl)	SGOT (U/L)
1	46	M	85	1.59	33.62	142	92	3	118	3.84	22.2	150	32	0.6	240	174	28	4.44	212	145	28
2	49	M	84	1.63	31.61	140	84	5	122.2	3.8	84.2	204	34	0.8	260	145	56	16.84	204	142	28
3	47	M	79	1.68	27.99	150	92	4	112	3.9	69.5	140	25	0.75	280	185	54	13.9	226	156	39
4	45	M	92	1.45	43.75	158	82	3	104.2	4.1	20.6	150	26	0.85	240	203	28	4.12	212	152	32
5	52	M	85	1.56	34.92	162	80	6	184	3.89	124	210	24	0.95	198	205	32	24.8	166	121	56
6	51	M	74	1.89	20.71	164	90	3	98.24	3.6	24	120	28	0.87	164	152	34	4.8	130	110	36
7	54	M	72	1.65	26.44	164	92	3	102.65	3.8	18.2	110	29	0.86	194	126	36	3.64	158	135	36
8	51	M	98	1.64	36.43	162	94	4	125	4.2	102.2	142	31	0.85	240	152	34	20.44	206	142	36
9	56	M	92	1.54	38.79	170	92	3	104.25	3.6	24.02	250	35	0.96	168	112	28	4.804	140	142	32
10	54	M	93	1.58	37.25	156	92	6	125	4.12	84	184	32	0.76	224	125	28	16.8	196	124	59
11	58	M	85	1.57	34.48	150	82	3	99.25	4.3	20.25	210	26	0.64	184	124	27	4.05	157	121	32
12	52	M	82	1.68	29.05	180	100	7	122.25	3.8	100.4	175	28	0.75	220	126	29	20.08	191	162	84
13	53	M	74	1.52	32.02	160	100	4	106.65	3.9	56.62	182	31	0.65	224	132	31	11.324	193	142	51
14	54	M	69	1.69	24.15	190	110	6	140	3.8	98.24	184	24	0.85	264	210	29	19.648	235	152	86
15	58	M	68	1.56	27.94	170	110	4	112.24	3.6	69.66	142	37	0.46	168	112	34	13.932	134	142	53
16	54	M	72	1.68	25.5	140	100	6	124.25	4.12	98.35	152	24	0.8	148	123	25	19.67	123	162	69
17	49	M	68	1.67	24.38	142	90	3	119.5	4.3	20.24	110	25	0.7	174	142	45	4.048	129	148	39
18	51	M	61	1.68	21.61	138	92	3	123.25	3.8	19.25	146	35	0.9	224	124	48	3.85	176	146	41
19	52	M	65	1.68	23.03	148	90	5	108.45	3.9	62.24	140	26	1.2	264	152	26	12.448	238	162	59
20	46	M	71	1.56	29.17	154	86	6	128.26	3.8	99.25	120	38	0.9	256	152	47	19.85	209	154	86
21	49	M	82	1.69	28.71	140	100	6	126.56	3.4	92.84	108	36	0.6	158	156	26	18.568	132	156	85
22	43	M	65	1.7	22.49	150	120	4	106.24	3.6	75.28	124	29	0.8	185	158	28	15.056	157	162	72
23	45	M	68	1.64	25.28	140	110	6	142.24	3.54	124.4	120	35	0.75	174	148	42	24.88	132	152	84

s.no	Age (yrs)	sex	Wt (kg)	Ht (mts)	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	Duration (hours)	IMA (U/dl)	S.ALB (g/dl)	CKMB (U/L)	RBS (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)	T-CHOL (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	LDH (mg/dl)	SGOT (U/L)
24	48	M	73	1.65	26.81	150	110	4	121.98	4.12	84.24	98	26	0.85	210	127	41	16.848	169	148	54
25	52	M	91	1.69	31.86	160	100	6	154.24	4.5	94.24	90	35	0.65	213	124	51	18.848	162	142	98
26	46	M	92	1.69	32.21	140	92	4	124	3.8	84.46	110	28	0.9	158	131	48	16.892	110	117	28
27	52	M	56	1.66	20.32	130	90	6	130.36	3.6	104.25	98	27	0.8	178	151	39	20.85	139	152	84
28	53	M	59	1.65	21.67	150	90	3	96.24	3.8	20.24	148	24	0.6	179	141	42	4.048	137	126	38
29	54	M	75	1.64	27.88	140	100	4	98.42	3.7	64.24	94	25	0.7	217	173	45	12.848	172	142	65
30	58	M	59	1.49	26.57	170	110	5	89.64	3.4	74.42	106	29	0.68	198	112	46	14.884	152	152	69
31	54	M	64	1.56	26.29	140	100	6	108.62	3.8	86.24	164	36	0.78	168	124	25	17.248	143	142	95
32	49	M	85	1.49	38.28	150	92	3	101.24	3.9	24	184	35	0.74	214	123	28	4.8	186	136	35
33	51	M	71	1.56	29.17	158	94	4	120.24	3.6	82.52	142	34	0.97	218	154	29	16.504	189	156	68
34	52	M	75	1.59	29.66	146	92	6	126.24	4.02	88.24	150	38	0.94	234	145	31	17.648	203	128	94
35	51	M	76	1.69	26.60	150	94	4	98.84	4.12	82.42	142	37	0.85	214	126	35	16.484	179	127	96
36	52	M	81	1.57	32.86	140	92	3	106.64	4.5	26.2	132	39	0.46	200	154	31	5.24	169	145	36
37	57	F	71	1.68	25.15	120	90	3	108.24	3.8	16.68	162	25	0.65	196	146	32	3.336	164	125	38
38	55	F	84	1.68	29.76	130	90	6	126	3.9	92.84	142	29	0.9	175	142	34	18.568	141	126	94
39	54	F	82	1.47	37.94	150	100	4	106.24	3.6	84.26	124	28	0.8	185	148	26	16.852	159	142	78
40	49	F	59	1.49	26.57	130	90	4	99.5	3.4	64.42	157	27	0.6	201	122	31	12.884	170	152	84
41	45	F	58	1.59	22.94	140	90	6	120.54	3.6	86.74	126	32	0.7	205	119	32	17.348	173	121	90
42	51	F	57	1.57	23.12	130	90	4	96.52	3.5	79.98	146	31	0.68	165	129	28	15.996	137	120	59
43	48	F	61	1.68	21.61	132	90	4	96.66	3.65	84.28	110	34	0.78	178	131	29	16.856	149	142	68
44	52	F	62	1.56	25.47	160	100	3	104.24	3.6	22.46	152	26	0.74	182	124	29	4.492	153	124	38
45	43	F	66	1.68	23.38	126	84	4	100.23	3.8	81.12	110	24	0.78	162	125	34	16.224	128	132	56
46	49	F	68	1.67	24.38	140	90	4	98.28	3.6	74.54	140	32	0.85	149	153	34	14.908	115	122	67
47	57	F	82	1.68	29.05	150	90	3	100.48	3.4	24.32	120	26	0.82	199	160	32	4.864	167	126	35
48	48	F	69	1.68	24.44	140	90	4	97.58	3.6	74.56	126	21	0.95	156	140	34	14.912	122	127	68
49	49	F	61	1.56	25.06	130	90	7	125.6	3.5	84.84	124	24	0.84	154	151	35	16.968	119	142	79
50	61	F	68	1.69	23.80	142	94	6	142	3.65	96.8	142	28	0.85	176	168	36	19.36	140	135	95

RESULTS AND STATISTICS:

In this study 50 patients were enrolled over an eleven months period from 94 eligible patients. The majority of the patients were male (74%) and the mean age was 51.13yrs . The mean age for females was 51.28yrs. The overall characteristics of the study group including the risk factors, biological variation etc are listed in table I. Among the data available on admission in the emergency department, factors associated with ACS were age , hyperlipidemia, smoking, hypertension. Positive family history and BMI.

CHARACTERISTICS OF THE STUDY POPULATION (TABLE I)

VARIABLE	ACS (NUMBER)	PERCENTAGE
Male	37	74%
Diabetes	19	38%
Hyperlipidemia	24	48%
Smokers	27	54%
Hypertension	35	70%
Family history	20	40%
BMI >25kg/m^2	35	70%

AGE DISTRIBUTION (Table II)

AGE	CONTROL (n=50)		STUDY (n = 50)		TOTAL (n = 100)	
41-50yrs	17	34%	19	38%	36	36%
51-60 yrs	31	62%	30	60%	61	61%
> 60 yrs	2	4%	1	2%	3	3%

Age Distribution (Fig 1)

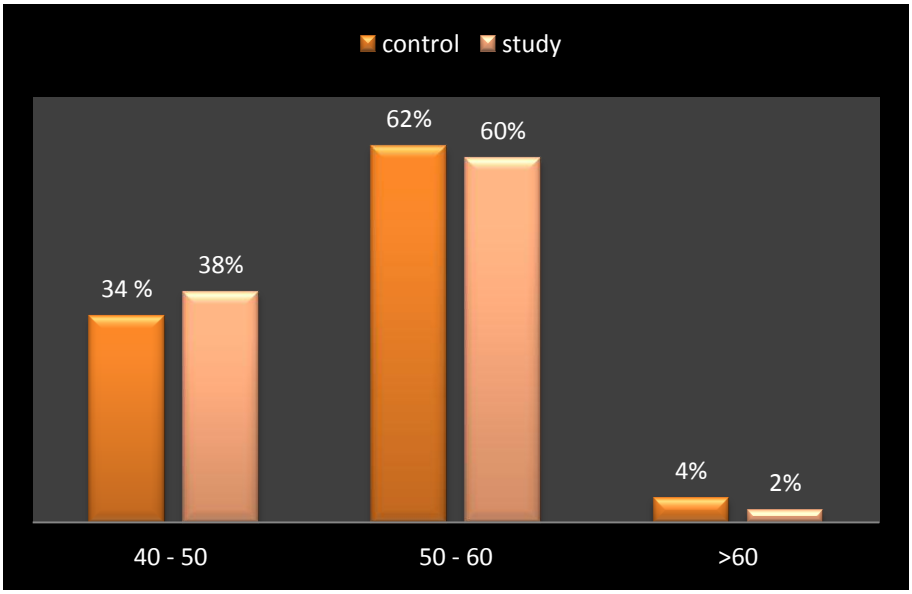


Table II shows the age distribution between control and study groups. The mean age falls between 51.88 ±4.51. All the patients included in the study were divided in three categories .

Group I 41 -50yrs → 17 → 34%

Group II – 51 -60 yrs → 31 → 62%

Group III - > 60yrs → 2 → 4%

GENDER DISTRIBUTION (Table III)

	CONTROL (n=50)		STUDY (n = 50)		TOTAL (n = 100)	
Male	37	74%	37	74%	74	74%
Female	13	26%	13	26%	26	26%

Gender distribution (both control and study) [Fig 2]

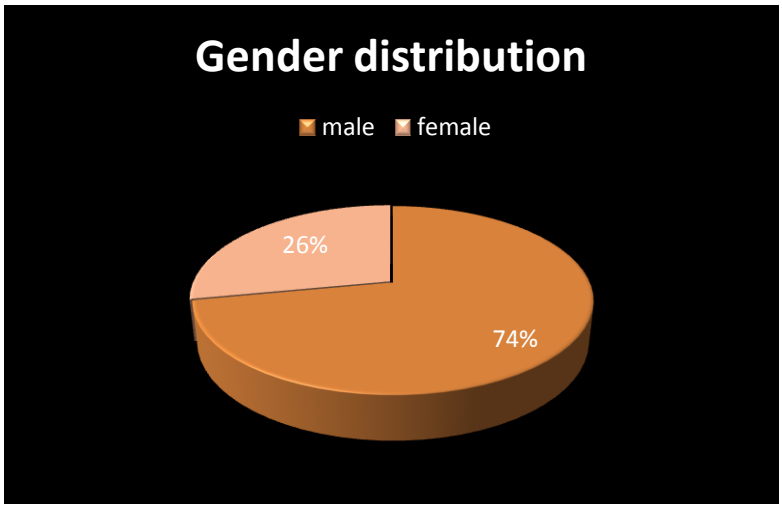


Table III shows gender distribution in both control and study group. Among the patients taken in this study 74% were male

BODY MASS INDEX (Table IV)

BMI	Category	Control (n=50)	Cases (n=50)
<18.5	Underweight	1	0
18.5 -24.9	Normal	12	15
25 - 29.9	Over weight	24	21
30-34.9	Obesity Gr 1	12	8
35- 39.99	Obesity Gr 2	1	6

	Mean	Standard deviation	Mean Rank	Sum of Ranks	Mann Whitney U score	Statistical Significance
Case	28.25	5.39	51.49	2475.5	1200.5	0.735
Control	27.24	4.03	49.51	2574.5		

BAR DIAGRAM:I

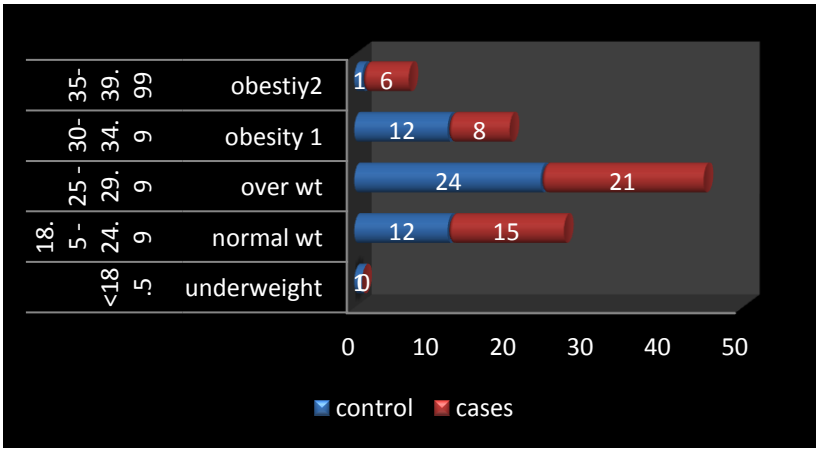


Table IV shows the distribution of BMI in the study group.

DESCRIPTIVE STATISTICS OF CONTROL GROUP (Table VI)					
	Number	Minimum	Maximum	Mean	Std. Deviation
AGE	50	43.00	61	51.88	4.51
WT	50	45.00	85	70.46	9.01
HT	50	1.450	1.89	1.61	0.08
BMI	50	16.23	36.03	27.24	4.02
SBP	50	110.0	142	126.92	8.19
DBP	50	70.00	90	79.76	4.17
IMA	50	10.60	61.24	37.60	14.74
S.ALBUMIN	50	3.400	4.5	3.79	0.26
CKMB	50	1.500	20.12	12.40	2.79
RBS	50	94.0	128	110.8	8.68
UREA	50	18.00	34	25.26	3.86
CREAT	50	.4600	1.2	0.82	0.13
T-CHOL	50	13.00	185	149.92	25.07
TGL	50	85.00	168	133.46	16.34
HDL	50	24.00	52	40.70	4.78
LDL	50	56.80	124.2	82.53	25.82
VLDL	50	17.00	33.6	26.69	3.27
LDH	50	15.00	168	108.04	34.28
SGOT	50	12.00	38	25.44	5.68

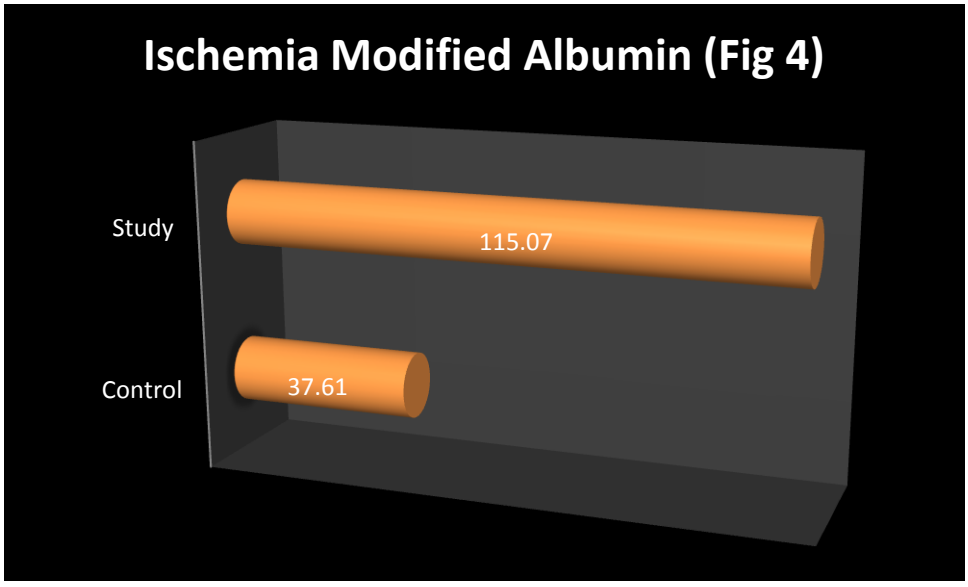
DESCRIPTIVE STATISTICS OF STUDY GROUP (Table VII)

	N	Minimum	Maximum	Mean	Std. Deviation
AGE	50	43	61	51.18	4.09
WT	50	56	98	73.78	11.07
HT	50	1.45	1.89	1.62	.08
BMI	50	20.32	43.75	28.25	5.38
SBP	50	120	190	148.28	14.26
DBP	50	80	120	94.28	8.21
DURATION	50	3	7	4.46	1.28
IMA	50	89.64	184	115.07	17.55
S.ALBUMIN	50	3.40	4.5	3.80	.27
CKMB	50	16.68	124.4	67.87	31.85
RBS	50	90	250	142.24	33.28
UREA	50	21	39	29.80	4.75
CREAT	50	.46	1.2	.78	0.13
T-CHOL	50	148	280	198.70	33.69
TGL	50	112	210	143.62	23.30
HDL	50	25	56	34.64	8.01
LDL	50	83.8	207.6	135.33	31.20
VLDL	50	22.40	42	28.72	4.66
LDH	50	110	162	139.58	13.69
SGOT	50	28	98	60.64	22.94

ISCHEMIA MODIFIED ALBUMIN (Table VIII)

IMA	Mean	SD	Mean rank	Sum of rank	Mann Whitney U score	*p* value
Control	37.61	14.74	25.5	1275	.000	.000 < 0.01 statistically significant
Study	115.07	17.55	75.5	3775		

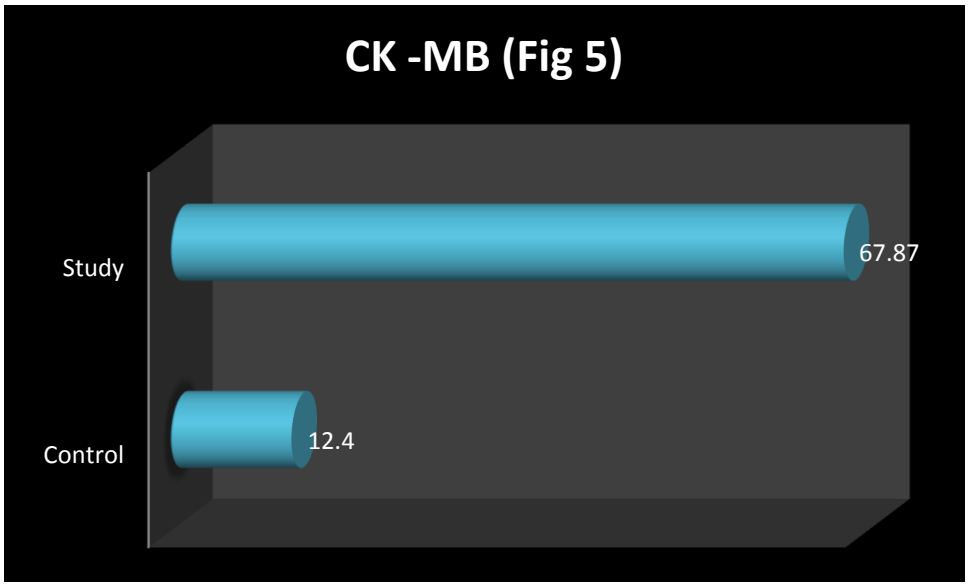
Table VIII show Mann Whitney test for comparing Ischemia Modified Albumin between control and study groups. The mean value of IMA for control and study group were 37.67±14.74 and 115.07±17.55 respectively . The Mann Whitney U score was .000 and p value .000 (<0.01)



CKMB (Table IX)

CKMB	Mean	SD	Mean rank	Sum of rank	Mann Whitney U score	*p* value
Control	12.40	2.79	25.56	1278.00	3.000	.000
Study	67.87	31.85	75.44	3772.00		

Table IX shows Mann Whitney test for comparing CKMB between control and study groups. The mean value of CKMB for control and study group were 12.40±2.79 and 67.87±31.85 . The Mann Whitney U score was 3.000 and p value.000 (< .01).



STATISTICAL ANALYSIS OF OTHER PARAMETERS BETWEEN CONTROL AND STUDY GROUPS (Table X)

	case	**60.64**	**22.94**	**73.92**	**3696.00**		
		Mean	SD	Mean Rank	Sum of Ranks	Mann Whitney U score	Exact significance (*p*)
S.ALBUMIN	control	3.79	0.26	50.00	2500.00	1225	0.864
	case	3.80	.27	51.00	2550.00		
RBS	control	110.8	8.68	34.28	1714.00	439	0
	case	142.24	33.28	66.72	3336.00		
UREA	control	25.26	3.86	37.16	1858.00	583	0
	case	29.80	4.75	63.84	3192.00		
CREAT	control	0.82	0.13	55.06	2753.00	1022	0.116
	case	0.78	0.13	45.94	2297.00		
T-CHOL	control	149.92	25.07	29.95	1497.50	222.5	0
	case	198.70	33.69	71.05	3552.50		
TGL	control	133.46	16.34	45.03	2251.50	976.5	0.059
	case	143.62	23.30	55.97	2798.50		
HDL	control	40.70	4.78	63.55	3177.50	597.5	0
	case	34.64	8.01	37.45	1872.50		
LDL	control	82.53	25.82	28.80	1440.00	165	0.059
	case	135.33	31.20	72.20	3610.00		
VLDL	control	26.69	3.27	45.03	2251.50	976.5	0.059
	case	28.72	4.66	55.97	2798.50		
LDH	control	108.04	34.28	35.76	1788.00	513	0
	case	139.58	13.69	65.24	3262.00		
SGOT	control	25.44	5.68	27.08	1354.00	79	0

Correlation between IMA and other parameters in study group (TABLE X)

IMA	Spearman Correlation rho value	*p* – values	Statistical Inference
S.ALBUMIN	0.230	0.108	Not significant
CK – MB	0.665	0.000	Significant
DURATION	0.639	0.000	
T- CHOL	0.185	0.197	Not Significant
TGL	0.228	0.112	
HDL	0.144	0.319	
VLDL	0.228	0.112	
LDL	0.131	0.363	
LDH	0.270	0.058	Significant
SGOT	0.355	0.011	

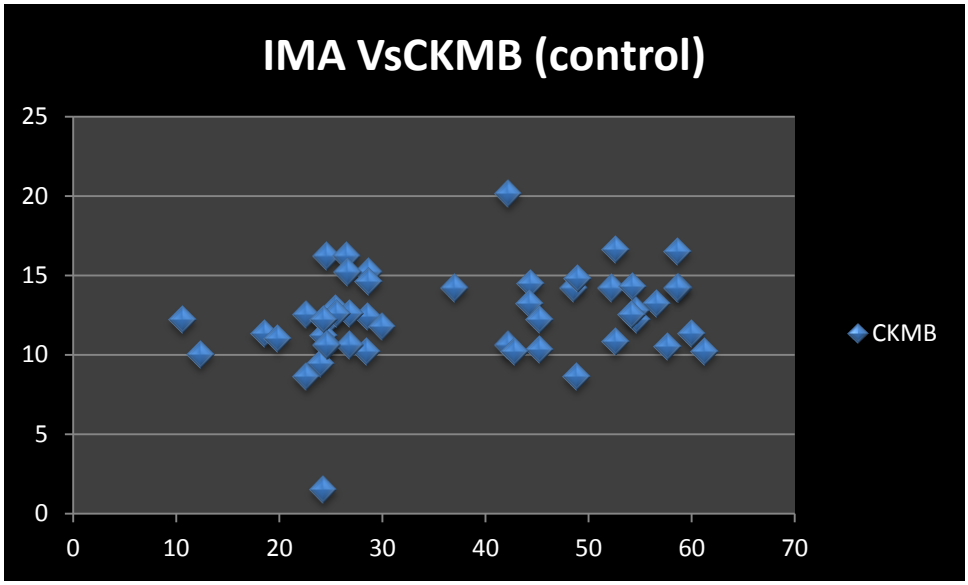
Table XI shows the correlation between IMA and other parameters in the study group. In this CKMB, LDH and SGOT showed significant correlation with IMA.

FREQUENCY TABLE FOR DURATION AFTER ONSET OF ACS AND SERUM LEVELS OF IMA AND CK-MB (TABLE XI)

		Hrs				Total	
		2 to 4 hours		4 to 8 hours		count	Col %
		count	Col %	count	Col %		
IMA (U/L)	Below 85	**2**	6.4	1	5.2	3	6
	Above 85	**29**	93.6	18	94.8	48	94
CK-MB(U/L)	Below 24	**12**	38.7	1	5.2	13	26
	Above 24	**19**	62.8	18	94.8	37	74

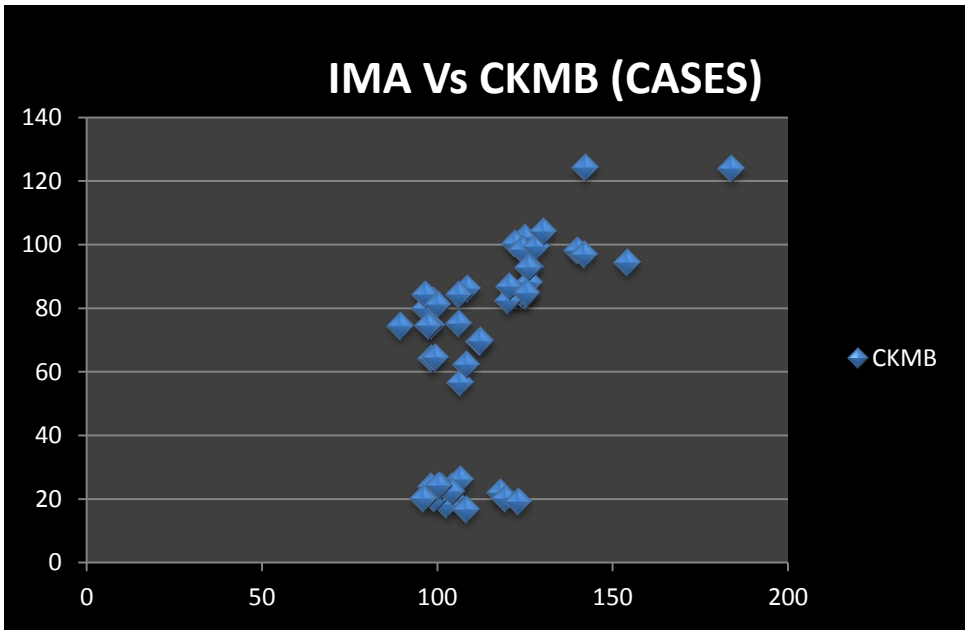
Table XI shows the comparison of time duration and rise of IMA and CKMB .

SCATTER DIAGRAM : I



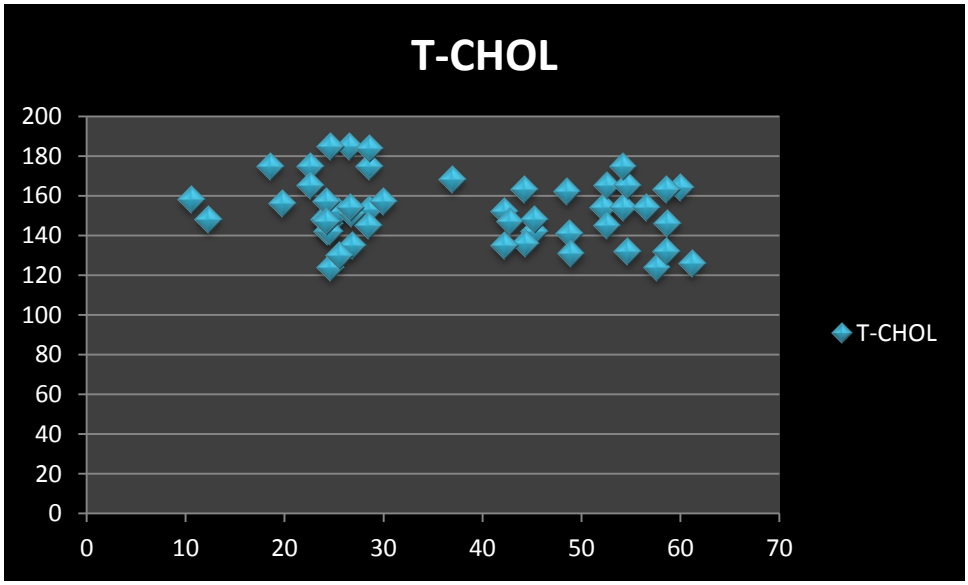
Scatter diagram I represents the correlation between serum Ischemia Modified Albumin and CKMB in control group

SCATTER DIAGRAM : II



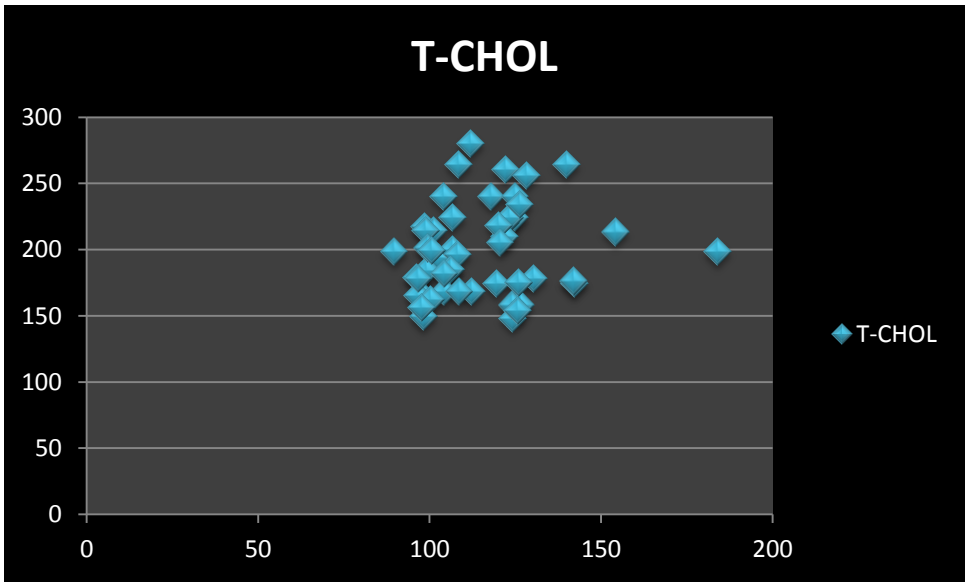
Scatter diagram II represents the correlation between serum Ischemia Modified Albumin and CKMB in study group.

SCATTER DIAGRAM : III



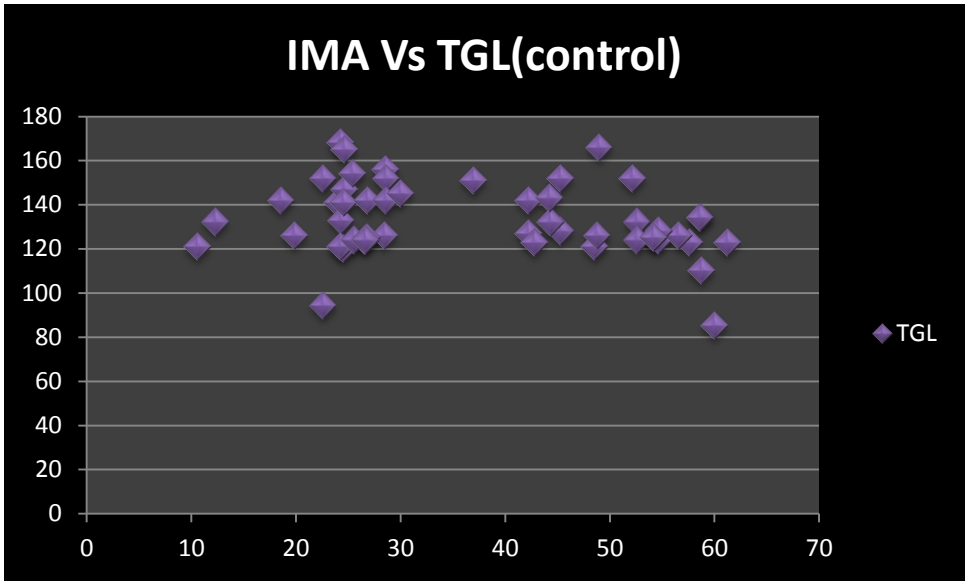
Scatter diagram III represents the correlation between serum Ischemia Modified Albumin and Total Cholesterol in control group.

SCATTER DIAGRAM : IV



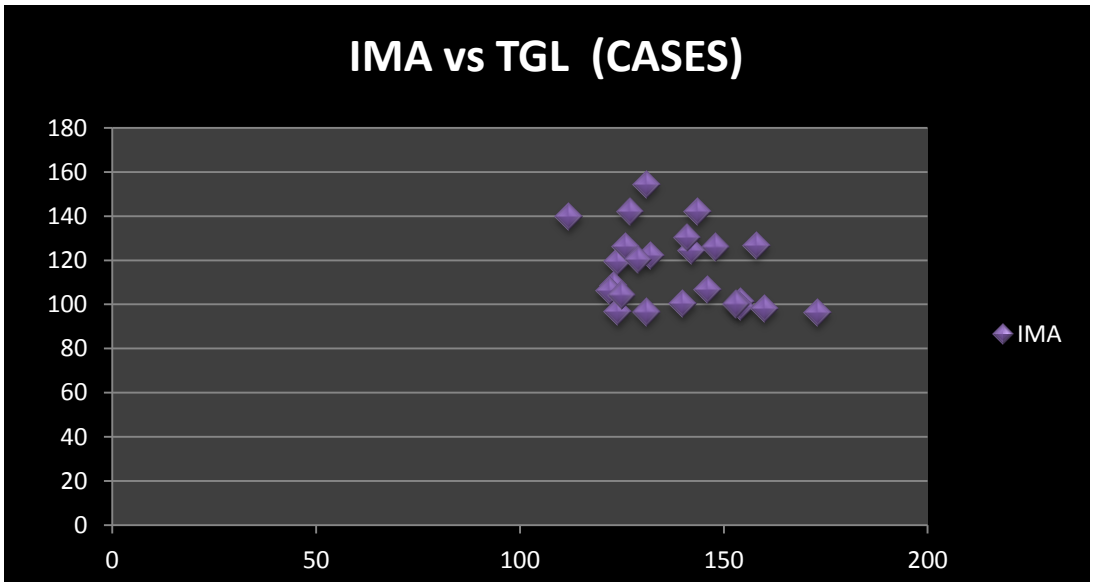
Scatter diagram IV represents the correlation between serum Ischemia Modified Albumin and Total cholesterol in study group.

SCATTER DIAGRAM : V



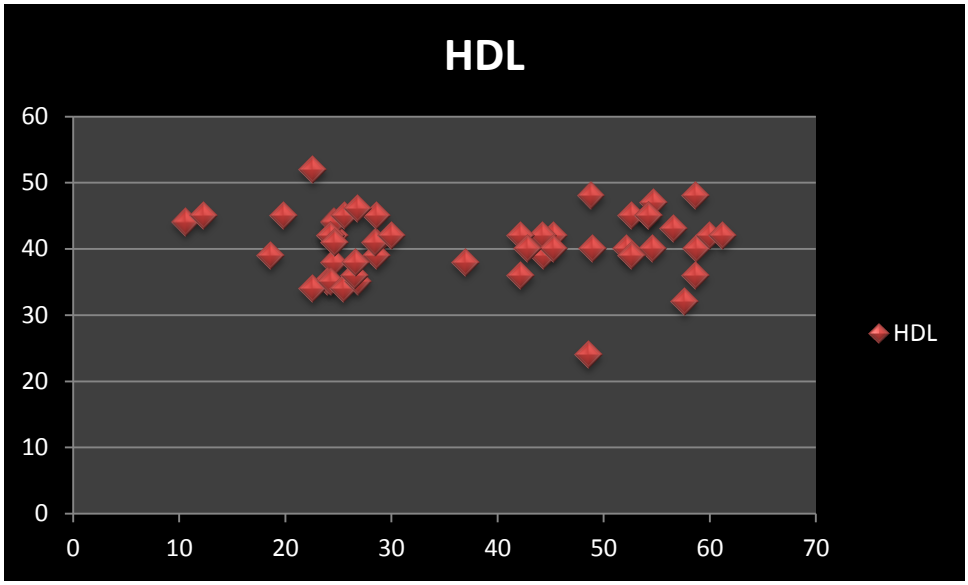
Scatter diagram V represents the correlation between serum Ischemia Modified Albumin and Triglycerides in control group.

SCATTER DIAGRAM : VI



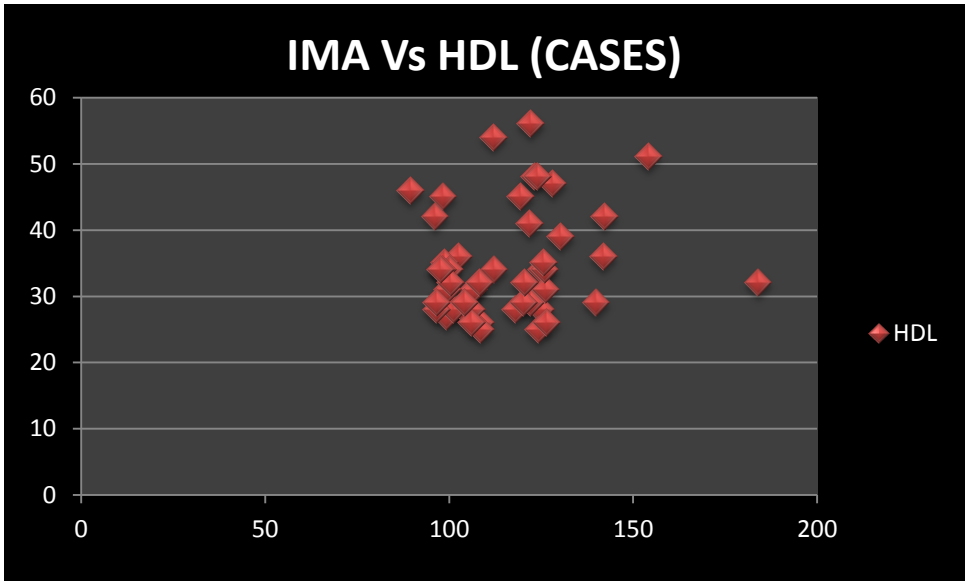
Scatter diagram VI represents the correlation between serum Ischemia Modified Albumin and Triglycerides in study group.

SCATTER DIAGRAM : VII



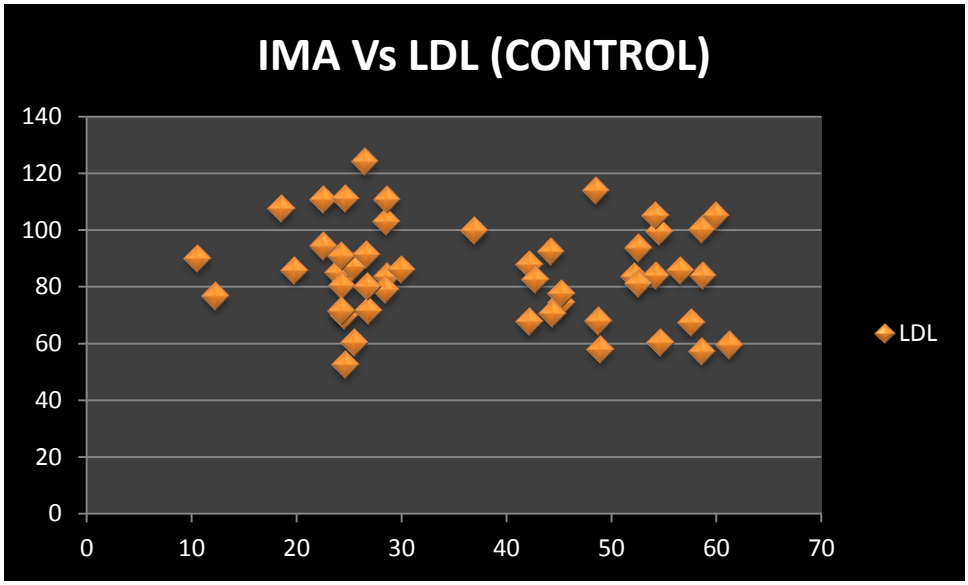
Scatter diagram VII represents the correlation between serum Ischemia Modified Albumin and HDL- cholesterol in control group.

SCATTER DIAGRAM : VIII



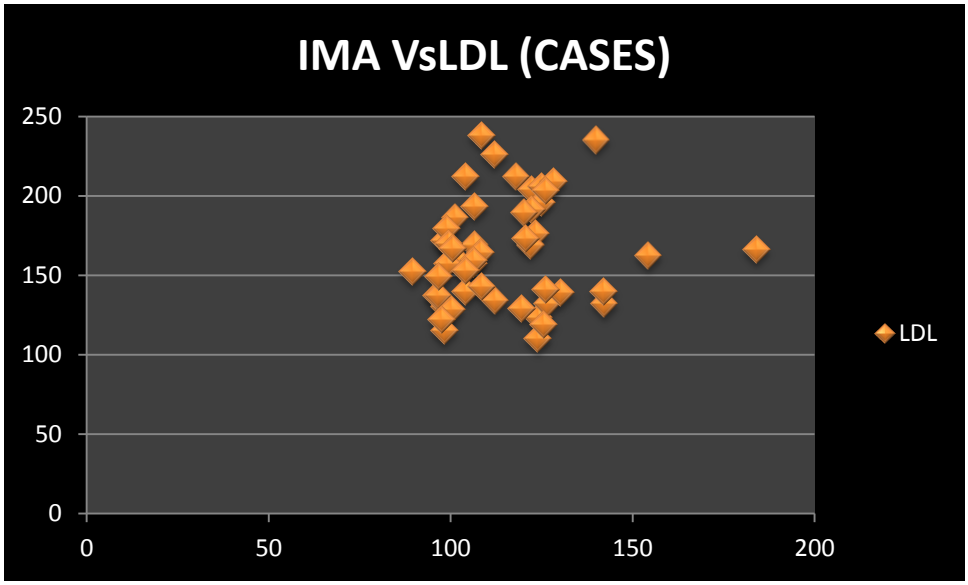
Scatter diagram VIII represents the correlation between serum Ischemia Modified Albumin and HDL - cholesterol in study group.

SCATTER DIAGRAM : IX



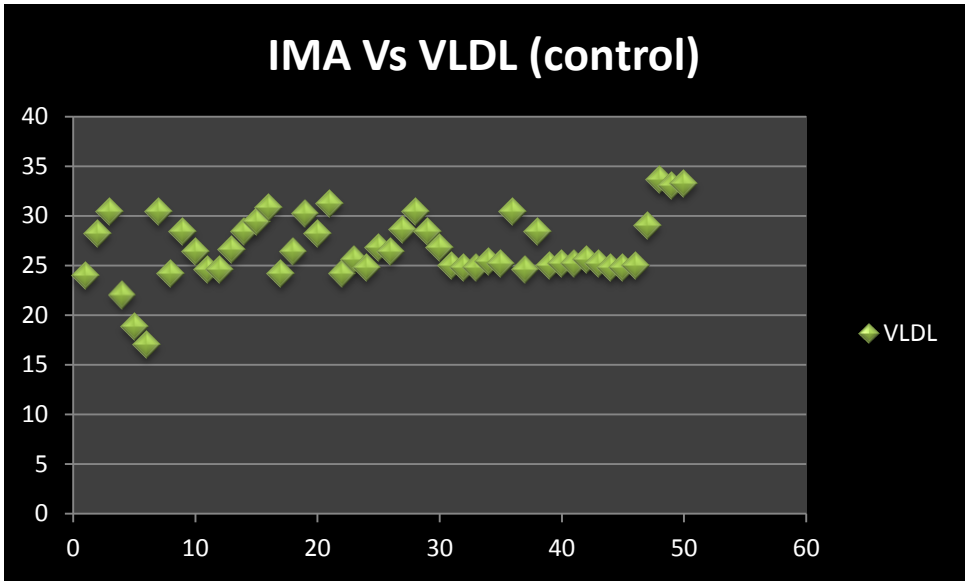
Scatter diagram VIII represents the correlation between serum Ischemia Modified Albumin and HDL - cholesterol in study group.

SCATTER DIAGRAM : X



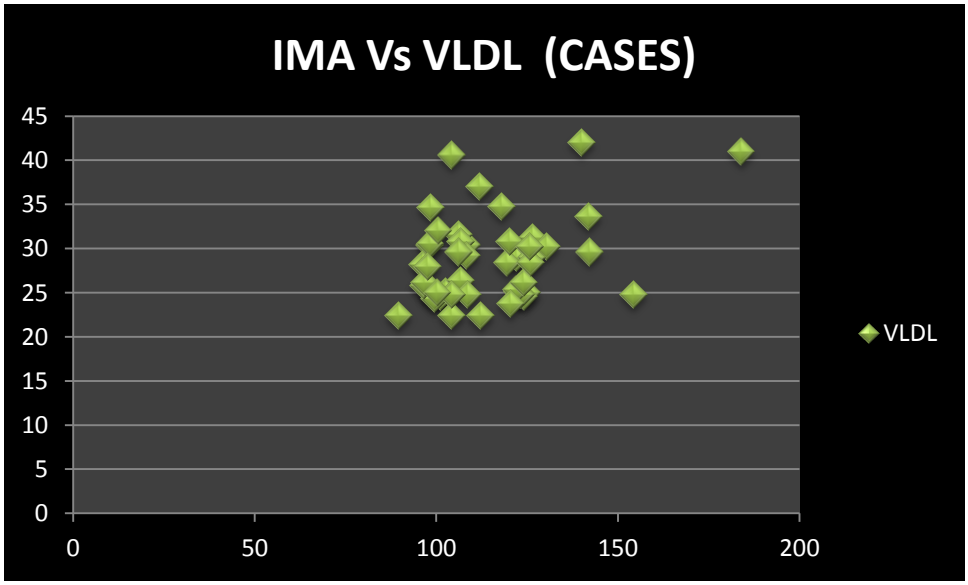
Scatter diagram X represents the correlation between serum Ischemia Modified Albumin and LDL- cholesterol in study group.

SCATTER DIAGRAM : XI



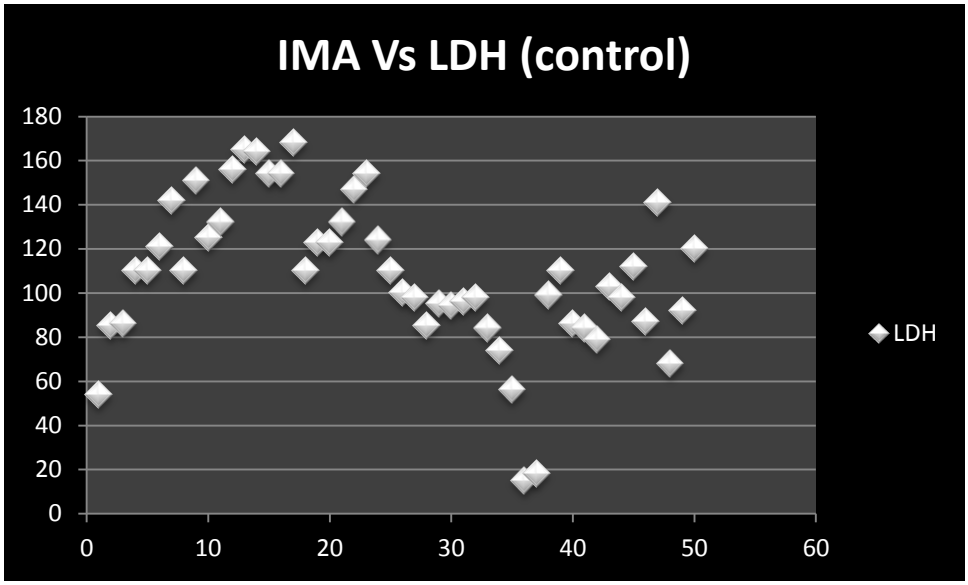
Scatter diagram XI represents the correlation between serum Ischemia Modified Albumin and VLDL in control group

SCATTER DIAGRAM : XII



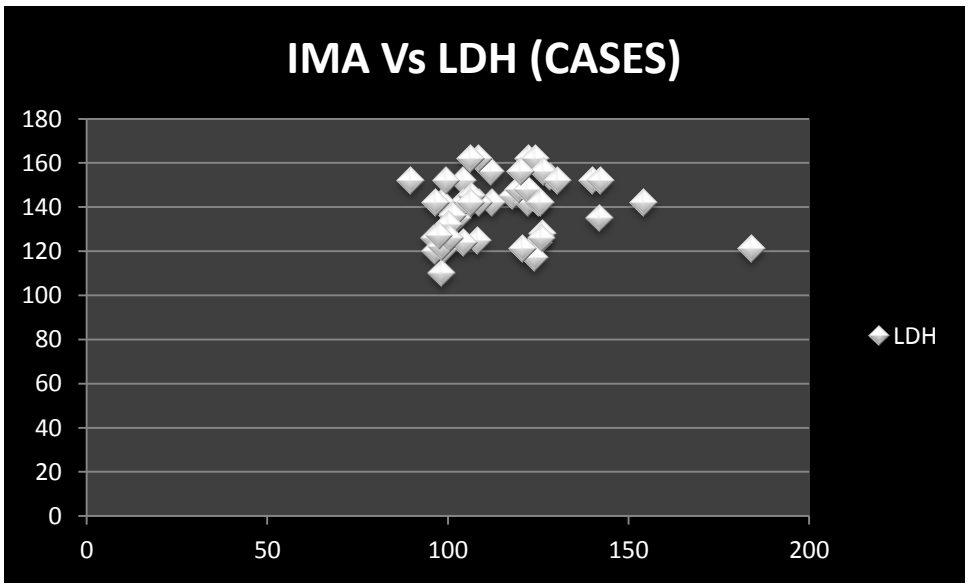
Scatter diagram XI represents the correlation between serum Ischemia Modified Albumin and VLDL in control group

SCATTER DIAGRAM : XIII



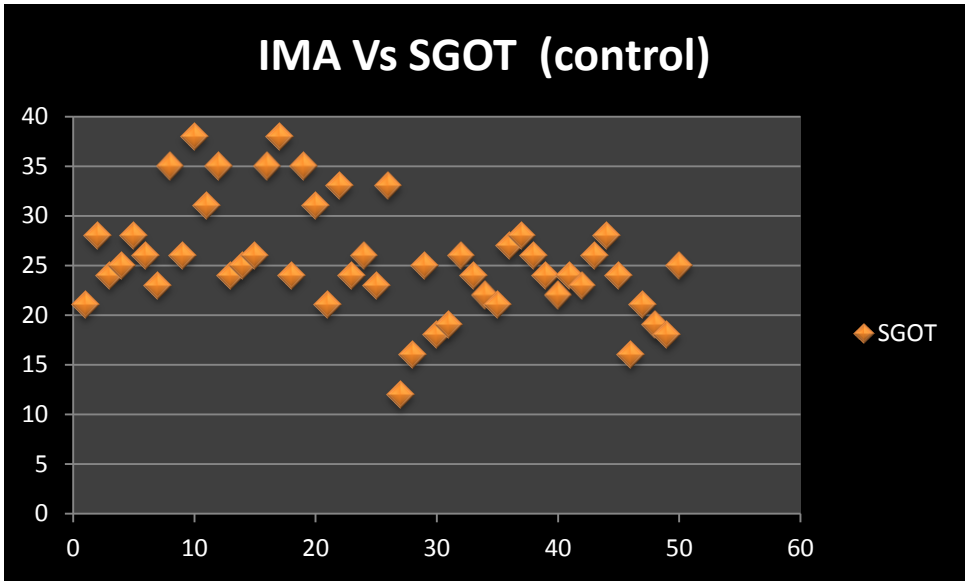
Scatter diagram XIII represents the correlation between serum Ischemia Modified Albumin and LDH in control group.

SCATTER DIAGRAM : XIV



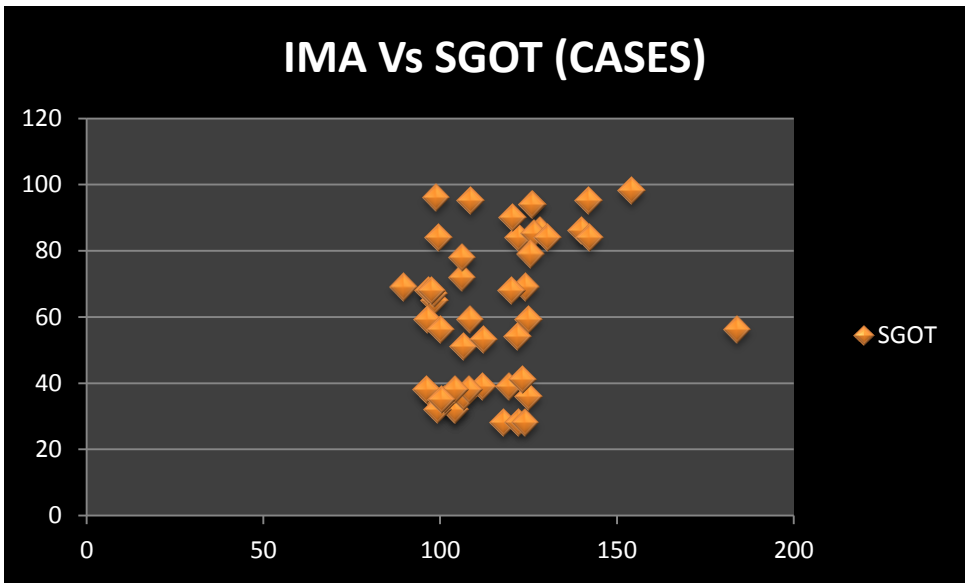
Scatter diagram XIV represents the correlation between serum Ischemia Modified Albumin and LDH in study group.

SCATTER DIAGRAM : XV



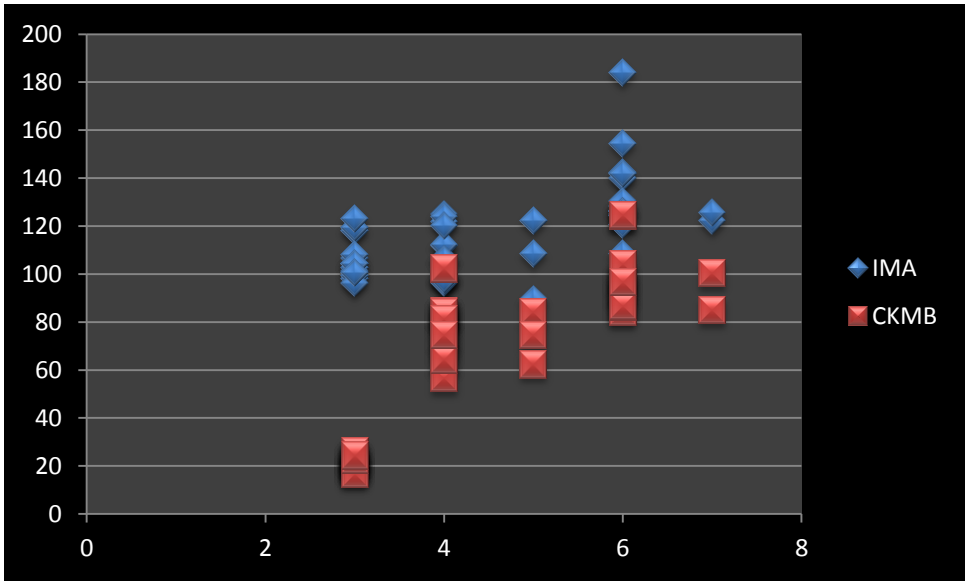
Scatter diagram XV represents the correlation between serum Ischemia Modified Albumin and SGOT in control group.

SCATTER DIAGRAM : XVI



Scatter diagram XVI represents the correlation between serum Ischemia Modified Albumin and SGOT in study group.

SCATTER DIAGRAM : XVII



Scatter diagram XVII represents the correlation between serum Ischemia Modified Albumin and time duration in study group.

DISCUSSION

Coronary heart disease accounts for increasing mortality and morbidity worldwide. Acute Coronary Syndrome is a subset which comprises of about 20-23% of patients presenting at emergency department.[80]

The diagnostic markers of AMI established by the world health organisation in 1986 included biomarkers as an integral part of the disorder and required at least two of the following criteris be met:

(1) a history of chest pain.

(2) evolutionary changes on the ECG.

(3) Elevations of serial cardiac markers to a level two times the normal value. However in over time , it became rare for a diagnosis of AMI to be made in the absence of myocardial injury.

A 2000 European Society of Cardiology / American College of Cardiology (ESC/ACC)[80] consenses conference updated in 2007 (Global Task Force) codified the role of markers by advocating that the diagnosis should be regarded as evidence of myocardial injury based on markers of cardiac markers in the appropriate clinical situation.[81] The guidelines thus recognised the reality that neither the clinical presentation for the ECG findings had adequate sensitivity and specificity. Many studies have proposed that combined use more than one cardiac biomarkers along with clinical findings and ECG.

The biomarkers for early identification of myocardial injuries both ischemia and necrosis in Acute Coronary Syndrome (ACS) are necessary in the diagnosis and the treatment decision to prevent the associated complications as well as to reduce the mortality. In the clinical practice, more attention has been paid to the estimation of serum levels of myocardial markers for the diagnosis of acute myocardial Ischemia, stratification of the ACS risk and the differential diagnoses of reversible versus irreversible myocardial ischemia and acute chest pain. The usefulness of biomarkers in ACS depends on the presence of myonecrosis. However , many patient with ACS may have myocardial ischemia without myonecrosis .Although currently available biomarkers sch as CK-MB, CK-MB mass trop-I , LDH, etc are useful in the diagnosis of ACS, they don't seem to increase before necrosis of myocytes and are time dependent. Moreover these markers to increase in the serum require cell death and leakage of proteins out of the myocytes which takes longer time for about 4 -6 hours.

Ischemia Modified Albumin is one of the recently idendified ischemic biomarker approved by U.S Food and Drug Admnistration. [82] Myocardial Ischemia changes the structure of the N-terminus of serum albumin which makes it unable to bind metals and can be measured by Albumin Cobalt Binding assay. This was found to increase in blood within minutes of the onset

of myocardial ischemia , remain elevated upto 6 -12 hours and return to normal within 24 hours.

In 2001 a multicentre study was done with 224 patients who arrived in the emergency department within 3 hours of the onset of symptoms suggestive of Acute Coronary Syndrome and the ability of ACB to predict positive and negative cTrop I result with 6 -24 hours after presentation. At the optimum cut off for the ACB test the sensitivity and specificity were 70% and 80% respectively, with a negative predictive value of 96%.

One study of patients with suspected ACS found that IMA had a better Negative Predictive value 92% than with combination of CKMB, myoglobin amd cTnT (86%). the sensitivity and specificity of elevated IMA for future mortality has been reported at 76% and 74%.

Shaoguing Juand et al., showed that the IMA concentration had increased significantly in the UA patients. In addition, IMA was negatively correlated with an abnormal Left Ventricular Ejection Fraction (LVEF), which was proved to be of clinically significant in the early diagnosis and the stratification of risk in patients with acute coronary syndromes.

In accordance with the above studies, in the study Ischemia Modified Albumin was increased earlier than CK-MB . The mean CK-MB level in the study group was (75.44±31.85) which is significantly higher than in the control group (25.56 ± 2.79) and found to be increased after 4 hours of onset of chest pain. But didnot show significant increase as IMA in the early hours of ischemia. The mean value of IMA of the study group (115.07 ± 17.55) was signicicantly higher than the control group (37.61 ± 14.74). Increase in IMA value and CK-MB was positively correlated in patients presented after hours. In this study , Ischemia modified albumin for the diagnosis of acute ischemic chest pain was significant along with other markers like increased CKMB and ECG findings. A biomarker of ischemia such as IMA will improve early diagnosis and also to rule out patients who do not have ACS and also more than 50% of patients presenting to the Emergency Department with chest pain were admitted to rule in or out Ischemic Heart Diease. Thus the serum levels of IMA can be used to both for diagnosis and rule in or rule out ischemic changes in the early hours.

The mean total cholesterol level in the study group (198.70± 33.69) was higher than the control group (149.92 ± 25.07) which was statistically significantly. This coincides with studies done by Mari Luomala et al and Lucie Locaste et al which suggested that total cholesterol more than 150mg/dl as risk factor for cardiovascular events.

The mean serum HDL –C which is lower in the study group compared to the control group (40.70± 4.78 verses 34.64 ± 8.01) which was statistically significantly ($p < 0.05$) and as per the recommendation of NCEP ATP III risk classification for HDL-C levels the serum HDL –C <4omg/dl considered as high risk for IHD.

The mean values of LDL-C, VLDL, and TGL are also significantly increased in the study group than the control group which contributes to increased risk for CHD.

Pearson correlation analysis showed significant correlation between IMA with CK-MB , LDH, SGOT and the duration within which it increases.

The strength of this study includes the homogenecity of the study group with respect to exclusion of possible confounding clinical conditions and proper timing of blood sampling. In this study mean value of IMA of study group was significantly higher than the control group and elevated within 2-3 hours. The mean value of CK-MB also significantly elevated but only after 4-6 hours and elevation of CKMB was well correlated with rise in IMA. Furthermore all the predisposing factors like smoking hypertension dyslipidemia , diabetes etc are present and significant number of patients are associated with these risk factors.

The measurement of IMA was done by simple cost effective chemical method (Albumin Cobalt Binding Test) and was approved by FDA. Elevation in IMA

identifies the early ischemic changes which will be reversible before irreversible necrosis occurs and are well correlated with CKMB levels also. The levels of IMA also was significantly higher than the healthy group and these results are in compliance with many literature data. Thus serum levels of Ischemia Modified Albumin can be used to identify ACS at an earlier stages which helps in Emergency Department to diagnose and to aid treatment decision.

CONCLUSION

Biochemical markers such as CK-MB, Cardiac Troponin-I and Myoglobin are suitable only for assessing myocardial infarction. The results of the present study confirm the findings of previous studies, that reported that the Albumin Cobalt colorimetric assay distinguishes myocardial ischemic patients from non ischemic patients ($p<0.001$)

IMA assay presents a quantitative accurate laboratory determination of the occurrence of an Ischemic myocardial event including angina of various types. Measurement of Ischemia Modified Albumin levels diagnose Acute Coronary Syndrome in patients with ongoing myocardial ischemia in Emergency Department. Measuring IMA along with ECG and other markers improves the diagnostic sensitivity of the method.

LIMITATIONS OF THE STUDY:

Further multicentre cohort study has to be done to assess the physicians in a better way in treatment decisions.

This assay need to be evaluated by incorporating it into decision making algorithm in an emergency department.

FUTURE SCOPE OF THE STUDY:

IMA level estimation in outpatient department itself can be done in patients complaining of chest pain to rule out myocardial ischemia before ECG manifestation especially in unstable angina, and other variants of Angina like Nocturnal Angina, prinzmetal's variant Angina and stable exertional Angina.

It can be also used to rule out non anginal causes of chest pain. If these observations are confirmed, IMA can be used as an outpatient investigation tool, to reduce in appropriate Hospital Admissions of Low risk patients.

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STUDY OF SERUM ISCHEMIA MODIFIED ALBUMIN IN ACUTE CORONARY SYNDROME –PROFORMA

NAME OF THE PATIENT :

AGE :

OCCUPATION :

ADDRESS :

COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

DRUG HISTORY :

GENERAL EXAMINATION:

Ht: Wt: BMI: BP: PR:

SYSTEMIC EXAMINATION:

CVS: RS:

ABD: CNS:

INVESTIGATIONS :

1.BLOOD SUGAR : FBS: PPBS:

2.SERUM ISCHEMIA MODIFIED ALBUMIN :

3.SERUM CK-MB

4.BLOOD UREA:

5.SERUM CREATININE:

6.LIPID PROFILE:

TOTAL CHOLESTEROL:

TRIGLYCERIDES:

HDL:

LDL:

VLDL:

SGOT:

LDH

CONSENT FORM

Dr .R.Freethi post graduate student in the department of Biochemistry, Thanjavur medical college, Thanjavur is doing a Study On Study of serum levels of ischemia modified albumin in acute coronary syndrome. The procedures has been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature :

Name:

Place: